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Total Synthesis of Enantiopure Biomolecules

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Chapter 4

β -D-Mannosyl Phosphomycoketides

4.1 Introduction

β -Mannosyl phosphomycoketides (MPM's) **4.1** and **4.2** are unusual mycobacterial antigens that were recently isolated in minute amounts by Moody and co-workers¹ from the cell wall of *Mycobacterium tuberculosis* and *Mycobacterium avium*, respectively (Figure 4.1). Initial structural characterization was based on degradation and mass spectrometric studies and did not include elucidation of the stereochemistry of the oligoisoprenoid chain.¹ In 2002, Dudkin and Crich reported the synthesis of **4.2**, confirming the overall molecular structure.² However, as a stereorandom alkyl chain (mixture of 32 diastereoisomers) was used, the stereochemistry of the lipid part remained unsolved.

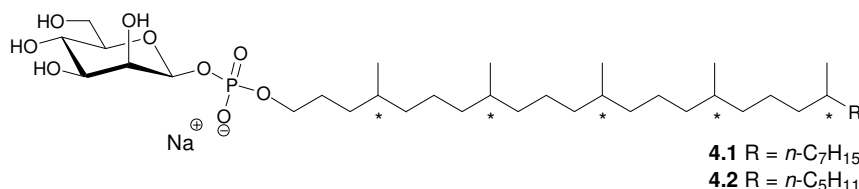


Figure 4.1 Molecular structure of β -mannosyl phosphomycoketides.

Structurally, **4.1** and **4.2** resemble a partially saturated β -mannosyl heptaprenyl phosphate isolated from *M. smegmatis* (Figure 4.2), that serves as a carrier of mycolic acid.³ However, an important difference from a biological point of view is that **4.1** and **4.2** violate the isoprene rule at both ends of their alkyl chain, which has implications for the biosynthetic pathway by which these saturated isoprenoid chains are assembled (see § 4.4).

From our perspective, **4.1** constituted a challenge to apply our procedure for the preparation of enantiopure saturated isoprenoid building blocks (Chapter 3) to a more complex target. Hence, we aimed to develop a general protocol for the construction of enantiopure MPM's with oligoisoprenoid chains of any desired stereochemistry and length.

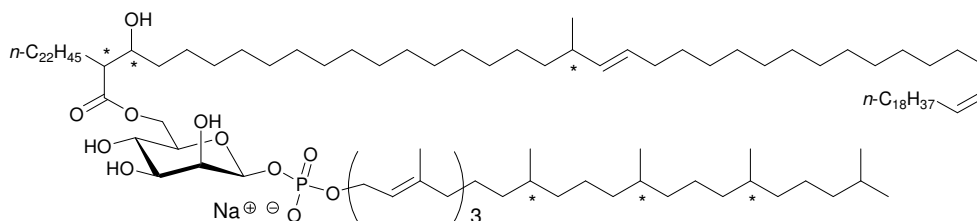


Figure 4.2 Related β -mannosyl heptaprenyl phosphate isolated from *M. Smegmatis*.³

4.2 Lipid-antigen mediated immune response

The success of the (human) immune system depends on its ability to distinguish foreign antigens from self-elements and to initiate appropriate effector mechanisms that destroy alien intruders without inflicting damage to self-elements.⁴ The mammalian immune system utilizes two major recognition strategies: innate and adaptive. Innate immune responses use pattern-recognition receptors such as lectins and the Toll-like receptors to recognize specific foreign molecules that are characteristic for microbial species. By focusing on conserved structural motifs that are common among pathogens, the innate immune system is able to respond immediately in case of infection. In contrast, the adaptive immune system uses recombinatorial antigen-receptors and is therefore able to interact with an almost infinite number of structures, although its response is generally slower. The main mediators of adaptive immune responses are T and B lymphocytes. While B-cells produce antibodies that can interact with epitopes of any molecular species, T-cells were believed until recently to be activated exclusively by peptides which are presented by major histocompatibility (MHC) class I and II proteins.⁵ In 1992, though, Porcelli *et al.* showed that a second class of antigen presenting proteins, known as the Cluster of Differentiation 1 (CD1) family, is capable of inducing proliferation of T-cells which are specific for microbial pathogens.⁶ Two years later they disclosed, that CD1 molecules have in fact the ability to present lipid antigens rather than peptides.⁷ Finally, in 2000, Moody *et al.* provided strong evidence that CD1-lipid complexes indeed play an important role in resistance to microbial infections *in vivo* via activation of T-cells.^{1,8} Since then, four members of the CD1 family (CD1a to CD1d) have been proven to present at least five classes of (glyco)lipids:⁹ mycolates,⁷ glycosphingolipids,¹⁰ phospholipids,¹¹ sulfoglycolipids¹² and lipopeptides.¹³ Based on

sequence similarities, the CD1 isoforms have been divided in two groups: CD1a, b, and c (group 1) and CD1d (group 2). The fact that many CD1d restricted T-cells express a semi-invariant T-cell antigen receptor (TCR) suggests that they may function as a pattern-recognition receptor of the innate immune system, while CD1a, b and c are part of the adaptive immune system.⁵

From a chemical point of view, lipids seem to be an unlikely target for recognition by T-cells. They are insoluble in the aqueous environment in which antigen-presenting molecules and T-cells function. Moreover, the high degree of conformational freedom of a long aliphatic chain and the absence of functional groups for hydrogen bonding, makes specific interactions with T-cell receptors (TCRs) unlikely. This paradox was partly resolved, when crystal structures of CD1 proteins showed the presence of large hydrophobic cavities.¹⁴ Based on these crystal structures and on investigations to establish the molecular determinants for antigen recognition,¹⁵ a model was proposed in which van der Waals interactions are maximized by embedding of the aliphatic chain(s) into the hydrophobic groove, while the polar headgroup is presented at the surface to interact with the TCR (Figure 4.3).^{4,14a}

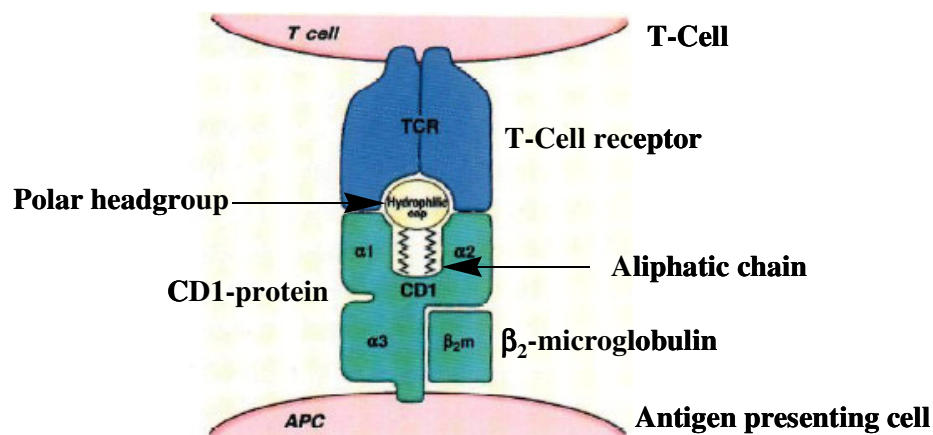


Figure 4.3 Trimolecular model of CD1-glycolipid antigen-T-Cell receptor interactions.⁴

4.2.1 CD1 proteins and lipid antigen presentation

Considering the limited α -amino acid sequence homology (< 36%) between CD1 proteins and MHC class I proteins, their three-dimensional structures are remarkably similar. Both antigen presenting molecules are integral membrane glycoproteins containing three domains ($\alpha 1$, $\alpha 2$ and $\alpha 3$), that form non-covalent heterodimers with β_2 -microglobulin (Figure 4.3).⁴ When evaluating tissue expression, CD1 proteins are more reminiscent of MHC class II proteins, in that they are mainly present on specialized antigen-presenting cells (APC's) like B cells, macrophages and dendritic cells. However, unlike MHC class I and class II proteins, human CD1 proteins are nonpolymorphic so that genetically unrelated individuals express nearly identical CD1 gene products. It is speculated that the overall lack of polymorphism of CD1 alleles is related to the limited structural variation in lipid tails of microbial species. In contrast to proteins, lipids and glycolipids are products of multi-enzyme biosynthetic pathways and their structures are therefore highly conserved.^{4,16} Moreover, (glyco)lipids are responsible for the integrity of the mycobacterial cell envelope, so that small changes in their structure are often detrimental for the survival of the bacterium.¹⁷ Incidentally, this conservation of structure makes these non-mammalian (glyco)lipids interesting biological targets for both drugs (such as isoniazid) and vaccines.

CD1 molecules are synthesized in the endoplasmic reticulum (ER), where N-linked glycans are attached and association with β_2 -microglobulin occurs.¹⁸ ER chaperones and self lipids subsequently assist proper folding,¹⁹ after which the CD1 complex is transported from the Golgi apparatus to the plasma membrane, presumably along the secretory pathway (Figure 4.4).²⁰ Surface CD1 proteins are then internalized, in case of CD1b and CD1c through the interaction of a tyrosine-motif in the cytoplasmic domain with adaptor protein complex 2 (AP2). Specific CD1 molecules are transported to particular intracellular compartments where the previously bound self lipids are exchanged for distinct foreign- or other self lipids. CD1a is predominantly targeted to recycling endosomes, CD1b is sorted to late endosomes and lysosomes, CD1c broadly traffics to all compartments and CD1d is found mainly in early and late endosomes.

The transport of lipids from hydrophobic environments like membranes and CD1 grooves to the aqueous lumens of intracellular compartments is a thermodynamically unfavorable process. Recent studies on the regulation of antigen presentation by human

CD1b and CD1d show that the loading of lipids is assisted by sphingolipid activator proteins (SAP), presumably through their interaction with the polar heads of lipids.^{9,21} The pathways that direct the CD1 molecules from the internal compartments back to the plasma membrane have not been elucidated yet.

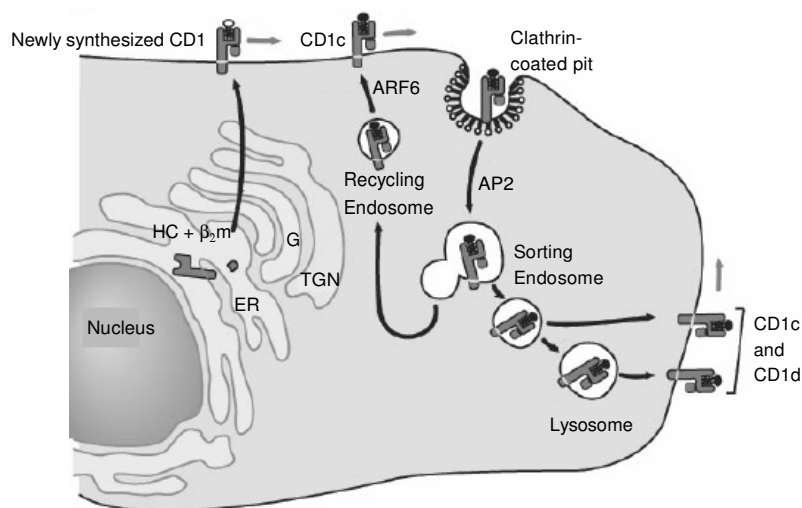


Figure 4.4 Intracellular trafficking and loading of CD1c and CD1d molecules.¹⁶

4.2.2 Recognition of CD1-lipid complexes by T-cell antigen receptors

T-cell antigen receptors (TCRs) that recognize peptide antigens bound in the groove of MHC molecules are heterodimers consisting of an α and a β chain. The specific interaction is mediated by the complementarity-determining residue (CDR) loops of the α and β chains which contact exposed residues of the peptide antigen and α -amino acids along the MHC α helices. The three-dimensional resemblance of CD1 molecules to MHC class I proteins (§ 4.2.1) allows the same type of α/β TCR heterodimers to specifically interact with CD1-lipid complexes as well. Indeed, the primary structure of TCRs that recognize CD1a, b and c in the presence of lipid antigens are indistinguishable from those that recognize MHC class I or II complexes with peptides.²²

In general, TCRs are highly specific for the epitope that is formed by the combination of a particular CD1 family member and a certain lipid antigen, even though there are some reports of self-recognition of CD1 in the absence of an antigen.²³ The molecular basis of the

CD1-lipid-TCR interaction awaits the determination of the structure of the tri-molecular complex (Figure 4.3). Frequent usage of basic α -amino acids in the CDR regions of the TCR suggests a model in which these CDR loops project directly between the CD1 α helices to participate in electrostatic interactions with the polar headgroups of the lipid antigens.²² In concurrence with this model, TCRs can distinguish even small changes in the structure of the hydrophilic headgroup of antigens,^{7,15,24,25} while alterations in the aliphatic tail often have less pronounced effects. Nevertheless, the influence of the aliphatic tail cannot be dismissed as inconsequential, since it often provides the (only) principal basis for discrimination of self- and non-self structures.²⁶

4.2.3 Effector functions of T-cells

In the peptide mediated system, class I MHC-peptide complexes can be found at the surface of infected cells, where they specifically bind to cytolytic T-cells having a CD8⁺TCR. Upon recognition, the cytolytic T-cells initiate an immune response which eventually leads to the lysis of the infected cells as well as killing of the invading pathogen. Class II MHC-peptide complexes on the other hand are presented at the surface of B cells, macrophages and dendritic cells where they specifically interact with T-helper cells (Th1/Th2) having a CD4⁺TCR. In this case, recognition results in the secretion of a variety of cytokines which stimulate the maturation, proliferation and activation of a range of different T-cells, B-cells, macrophages and so on. *De facto*, a cell is either killed or proliferated depending on the presented MHC-peptide complex and a strict separation of these activities is obviously instrumental for the proper functioning of the immune system.

In this light, it is noteworthy, that group 1 CD1-lipid complexes are recognized by both T-cells expressing a CD8⁺TCR as well as by T-cells having a CD4⁺TCR among others. To make the situation even more complex, some CD1-restricted T-cells display both effector functions characteristic for cytolytic T-cells as well as features of T-helper cells.⁵ Indeed, the same CD1-restricted T-cell that is involved in the lysis of infected cells and in direct antimicrobial activity *via* the secretion of perforin and granulysin, respectively,²⁷ can also be responsible for the production of interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α), which activate the microbicidal functions of macrophages (Figure 4.5).^{5,16} How the lipid-based immune system distinguishes infected cells, which have to be lysed, from B

cells, macrophages and dendritic cells, which have to be proliferated, remains to be elucidated.

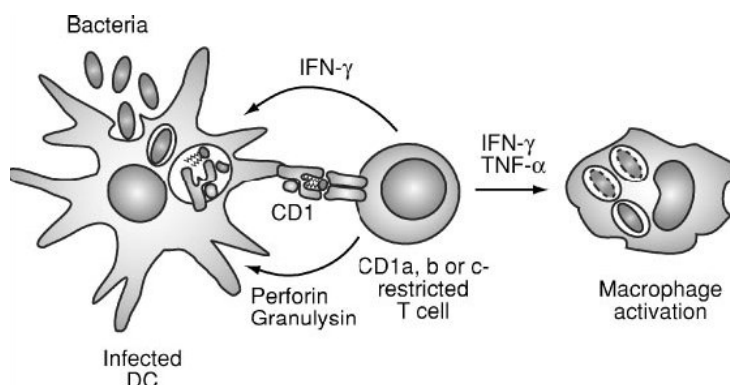


Figure 4.5 Effector functions of CD1-restricted T cells.¹⁶

4.2.4 Rationale for a lipid-antigen mediated immune response

The rationale for a lipid-antigen mediated immune response is still a subject of debate. It may be that the lipid-antigen mediated pathway serves to identify infected cells that inefficiently present protein antigens, thus providing a complementary mechanism.⁵ In addition, CD1 molecules are expressed on immature dendritic cells, suggesting that CD1-restricted T-cells may function at an earlier phase of the immune response than MHC-restricted T-cells.⁵ Finally, evasion of immune responses mediated by protein antigens may readily occur by point mutations that do not affect protein function, while (glyco)lipid structures are highly conserved (*vide supra*). However, by definition, the tailor-made system for an instant immune response against antigens with a common motif is the innate immune system. Hence, the first explanation for the existence of an adaptive immune system based on (glyco)lipids seems to be more plausible than the latter two arguments.

4.3 CD1c-mediated T-cell recognition of isoprenoid glycolipids

In 2000, it was reported that CD1c-presentation of two previously unknown β -D-mannosyl phospholipids (4.1 and 4.2) isolated from *Mycobacterium tuberculosis* resulted in recognition by T-cells (CD8-1) expressing a CD8⁺TCR.¹ Notably, this was the first

example of a lipid antigen having a single alkyl chain instead of the more common motif of two straight aliphatic hydrocarbon tails connected by a central hydrophilic cap. To gain further insight into the molecular determinants that affect recognition, a range of semi-synthetic glycosyl- β 1-phosphoisoprenoid compounds were prepared and tested. As predicted by the tri-molecular TCR-CD1-antigen model (§ 4.2.2 and Figure 4.3), the outcome demonstrated a fine specificity for the hydrophilic headgroup. Both removal of the mannose or exchange for glucose resulted in a complete loss of activity. In addition, a saturated α -prenyl unit was required, but both *cis* and *trans* double bonds elsewhere in the alkyl chain were tolerated. Finally, T-cell response appeared inversely proportional to prenyl length. A glycolipid with an alkyl tail of 35 carbons responded stronger than one with a C55-tail, while the analogue with a C95-tail was completely inactive.

Since the above study was performed using living cells rather than immobilized CD1 proteins, it is unclear what is causing changes in TCR recognition. It could be that changes in the structure of the lipid antigen result in different affinity for the CD1 protein or that the CD1-antigen complex interacts differently with the TCR. However, it is also possible that modification of the lipid antigen influences intracellular trafficking and delivery of the glycolipids to relevant antigen-loading compartments. Likewise, it is also uncertain whether the fact that only CD1c presents lipid antigens with a single alkyl chain is due to specialization of the CD1c groove or to the loading pathway of the antigen. Related to this, the dilemma remains how the immune system discriminates between self and foreign mannosyl- β 1-phosphate lipids.²⁸ Even though a mannosyl- β 1-phosphate moiety with a saturated α -prenyl unit and a short tail seems both necessary and sufficient for recognition, the saturated isoprenoid chain of β -D-mannosyl phosphomycoketides has to be the key element responsible for selectivity. To obtain insight into the subtle mechanisms that underlie selectivity, complementary information should be acquired by *in vitro* and *in vivo* tests. A necessity for such experiments is the availability of sufficient and pure (glyco)lipid antigens of any desired composition. Considering the slow growth of *M. tuberculosis* and the minute amounts of β -mannosyl phosphomycoketide that it produces, total synthesis seems to be the only way to fulfill this requirement at present. Assembly by total synthesis has the additional advantage that non-natural antigens are accessible as well.

4.4 Biosynthesis of mycoketides²⁶

In the original paper on CD1c-mediated T-cell recognition of isoprenoid glycolipids the authors commented on the molecular structure of the antigens (**4.1** and **4.2**) in the following way: ‘Unusual features of the newly discovered mycobacterial isoprenoids (...) included the complete saturation of their alkyl chains, and a deviation from the repeating five carbon prenyl unit at the proximal and distal ends of these chains.’¹ Since the structure of the saturated alkyl chain could not be explained by a conventional C₅ isopentenyl pyrophosphate biosynthetic pathway, an investigation was started to determine the genetic and enzymatic basis of **4.1** and **4.2**. Metabolic labeling and mass spectrometric analysis suggested an elongation mechanism using alternating C₂ and C₃ units, which is indicative for a polyketide synthase pathway. Inspection of the *M. tuberculosis* genome identified one candidate gene, *pks12*, which was predicted to encode a large protein consisting of 12 contiguous but separately functioning catalytic domains. Knockout and complementation of *pks12* established that this gene was indeed responsible for the production of the saturated isoprenoid tails of **4.1** and **4.2**. As a consequence, **4.1** and **4.2** were thereafter referred to as mannosyl phosphomycoketides (MPM) rather than isoprenoids. Interestingly, mannosyl-β-1-phospholipids with true polyisoprenol lipid moieties, with their biosynthesis not disrupted by *pks12* genetic deletion, did not substitute as potent antigens for the T-cell response.

During the elucidation of the biosynthetic pathway, MPM's with a tail length of 31, 33 and 34 carbons were observed next to the already known species with 30 (**4.2**) and 32 (**4.1**) carbons. A plausible polyketide based mechanism for the biosynthesis of all these structurally related mycoketides starts from different carboxylate primers of varying length. Subsequently, (two) acyltransferases and (two) ketosynthases add malonate (C₂) and methylmalonate (C₃) units in an alternating fashion. After every C₂- or C₃-addition, the consecutive action of a ketoreductase (to the alcohol), a dehydratase (to the alkene) and an enoylreductase provides the saturated system (see Scheme 2.1). Since C₂-addition is followed by C₃-addition and vice versa rather than at random, it is likely that two sets of catalytic domains are required, one for malonate units and one for methyl malonate units. The stereochemistry of the five asymmetric centers is determined by the enoylreductase

following C₃-addition. The final carboxylate moiety has to be reduced to the alcohol and phosphorylated to yield a substrate for glycosylation.

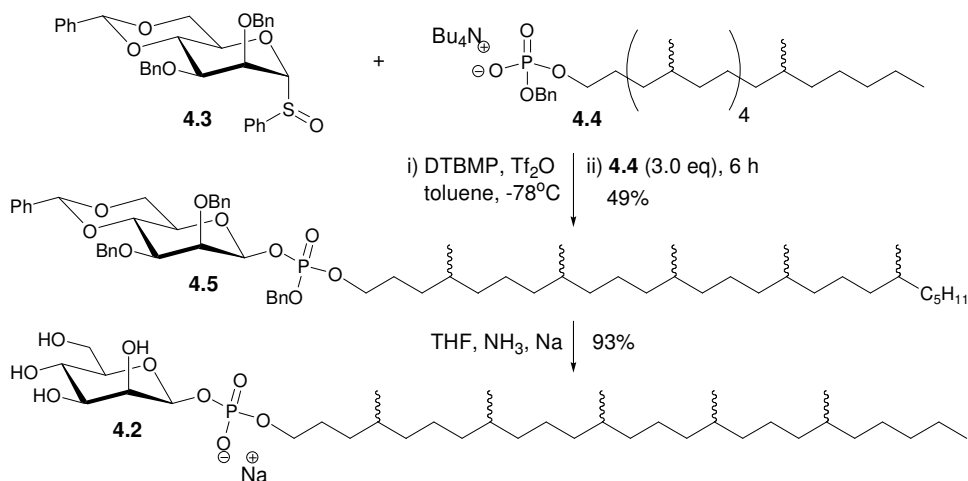
The unraveling of the biosynthetic pathway of MPM's has raised speculations regarding their biological function. Their complex biosynthesis is energetically expensive and conserved in infectious mycobacteria while lacking in non-virulent mycobacteria. This suggests that they may have a highly specialized role that is essential for intracellular growth. In agreement with this hypothesis, MPM's are found at low absolute levels; they make up about 1 ppm of the total weight of the cell wall. Possibly MPM's are involved in mannose transmembrane transport or transfer.²⁶

4.5 Synthesis of stereorandom β -D-mannosyl phosphomycoketides

The central theme in the synthesis of β -D-mannosyl phosphomycoketides by Crich and Dudkin,² was the formation of the β -mannosyl phosphate linkage. As a result of steric repulsion caused by the 1,2-*cis* configuration and instability due to the anomeric effect, β -mannosyl glycosidic linkages in general are notoriously difficult to bring about.²⁹ However, in a series of publications, Crich *et al.* disclosed an elegant method for the direct formation of such linkages.³⁰ Their procedure involves *in situ* conversion of a 4,6-*O*-benzylidene protected α -mannosyl sulfoxide (for example **4.3**; Scheme 4.1) into the corresponding α -anomeric triflate. Subsequent addition of an acceptor leads to S_N2 type displacement to give the desired β -mannoside with high selectivity.³¹ The origin of this high selectivity lies in the torsional disarming properties of the 4,6-*O*-benzylidene moiety, which opposes flattening of the molecule and thus prevents the formation of the oxocarbenium ion necessary for S_N1 type reactions.³²

In order to construct MPM **4.2**, the general mannosylation protocol discussed above was applied to the coupling of sulfoxide donor **4.3**, which was prepared in six steps from D-mannose, to phosphate acceptor **4.4** (Scheme 4.1). Since formation of the β -D-mannosyl phosphate linkage was the main goal of the investigation, a stereorandom mixture of **4.4** was used consisting of 32 stereoisomers. In the event, **4.3** was reacted with Tf₂O (1.1 eq) in toluene at -78 °C in the presence of acid scavenger 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), followed by the addition of three equivalences of **4.4** to give the desired product

(**4.5**) in 49% yield after six hours. Subsequent deprotection *via* Birch reduction (Na , NH_3) gave the final product **4.2** with exclusively the β -configuration in 93% yield.



Scheme 4.1 Synthesis of racemic β -D-mannosyl phosphomycoketide **4.2** by Crich *et al.*²

4.6 Total synthesis of enantiopure β -D-mannosyl phosphomycoketides

To study the effect of the stereochemistry of the saturated alkyl chain on the activity of MPM's, a synthetic protocol that is capable of accessing any desired diastereomer is required. Hence, we aimed at the development of a general (catalytic) strategy using enantiopure saturated isoprenoid building blocks (see chapter 3) as key intermediates.

As explained in § 4.4, the stereochemistry of natural phosphomycoketides is determined by an enoylreductase following the introduction of methylmalonate. Provided that a single catalytic domain is responsible for this transformation, the product most likely has an all-*syn* configuration. Since the authors, who elucidated the biosynthetic pathway, predicted the stereochemistry to be all-S (private communication), we decided to direct our initial efforts to the preparation of this diastereomer of **4.1**.

4.6.1 Retrosynthesis

When we started the synthesis of enantiopure MPM's, we envisioned that three requirements had to be fulfilled in order to prepare useful amounts of final product. First of

all, since connection of two saturated isoprenoid units (**A** and **B** in Figure 4.6) would generate an alkyl chain with four stereogenic centers, an additional (catalytic asymmetric) method had to be found for the introduction of the fifth asymmetric center (**C**). Conveniently, the catalytic asymmetric conjugate addition of Grignard reagents to α,β -unsaturated thioesters was under investigation in our group at that moment and we planned to employ this procedure to obtain fragment **C**.³³ Secondly, as the various linear chiral building blocks (**A**, **B** and **C**) are relatively valuable, an efficient procedure had to be found to cross-couple them without using a large excess of either coupling partner. Related to this, formation of the β -D-mannosyl phosphate linkage as described by Crich and Dudkin was undesirable,^{2,30g} since it would require three equivalents of precious oligoisoprenoid chain. We therefore intended to either adapt their methodology in such a way that an excess of sulfoxide donor **4.3** could be used or to employ an entirely different coupling strategy.

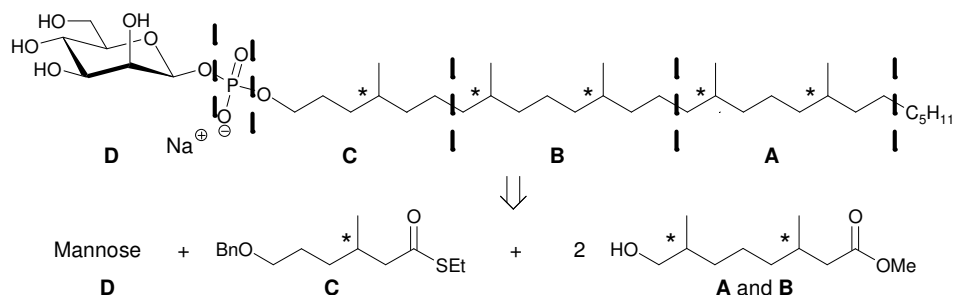


Figure 4.6 Retrosynthetic analysis of β -mannosyl phosphomycoketide **4.1**.

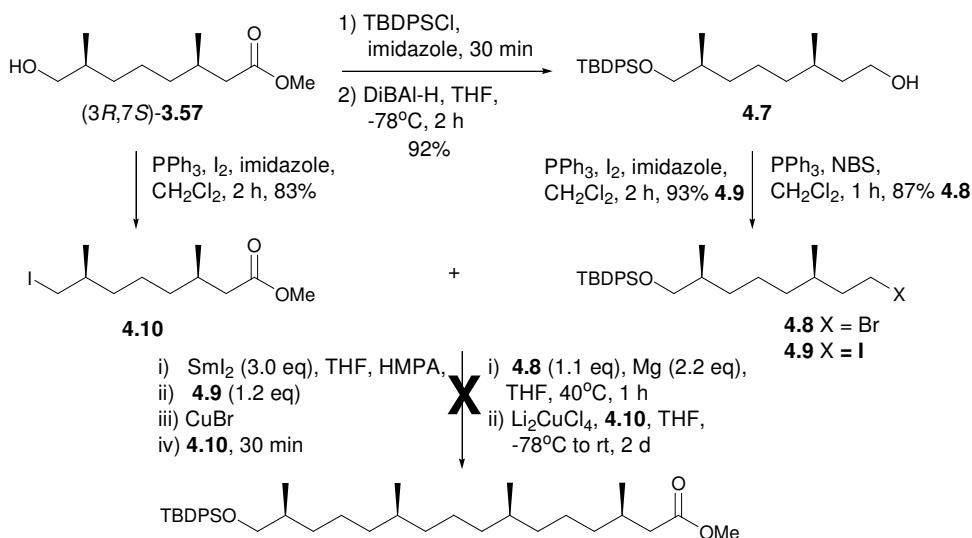
4.6.2 Synthesis of diisoprenoids

The direct formation of sp^3 - sp^3 carbon bonds in the presence of other functionalities, like for example (protected) alcohols or esters, is still a difficult transformation in organic synthesis. This particularly holds true when a large excess of either coupling partner has to be avoided, like in the synthesis of an expensive homo-chiral saturated isoprenoid chain. The classical method to cross-couple isoprenoids in order to obtain saturated diisoprenoids relies on an indirect approach. It consists of a S_N2 reaction between an α -deprotonated alkyl sulfone and an alkyl halide followed by a metal reduction (see scheme 3.1).³⁴ However, the reductive removal of the sulfone proceeds under harsh conditions, that preclude the presence of, for example, esters. Moreover the yield in this second step is somewhat

unreliable as it ranges from modest to good (50-85%). For the connection of our linear fragments, we decided to search for a milder and less capricious direct or indirect coupling procedure.

4.6.2.1 Copper-catalyzed Grignard and SmI_2 -assisted cross-couplings

In 1997, Berkowitz and Wu described the direct formation of saturated diisoprenoids *via* copper-catalyzed cross-couplings using Grignard and samarium chemistry.³⁵ Our attempt to reproduce their results started with the protection of the hydroxyl moiety of (3*R*,7*S*)-**3.57** *via* reaction with *tert*-butyldiphenylsilyl chloride (TBDPSCI) in the presence of imidazole (**4.6**, 95%, Scheme 4.2). The methyl ester was then reduced with DiBAL-H to afford primary alcohol **4.7** (97%). Subsequent treatment of **4.7** with either triphenylphosphine and NBS or with PPh_3 , iodine and imidazole furnished primary alkyl halides **4.8** (87%) and **4.9** (93%), respectively. In an analogous fashion, (3*R*,7*S*)-**3.57** was converted into primary iodide **4.10** in 83% yield.



Scheme 4.2 Preparation of diisoprenoids using Grignard/samarium cross-coupling.

Disappointingly, Li_2CuCl_4 -catalyzed Grignard coupling of **4.8** to **4.10** did not result in the formation of the saturated diisoprenoid in our hands (lit.³⁵ 71%). Furthermore, SmI_2

mediated cross-coupling of **4.9** and **4.10** in the presence of HMPA and catalytic copper bromide (20 mol%) was unsuccessful as well (lit.³⁵ 81%).

Careful optimization of the SmI_2 -mediated reaction using comparable (non-chiral) compounds eventually gave yields of up to 40% in model reactions, while the isolated yields with magnesium were even lower. In the latter case, the problems were partly due to inefficient formation or instability (degradation and/or homocoupling) of the Grignard reagent. Titration with *sec*-butanol in the presence of 1,10-phenanthroline revealed that approximately only 60% of the alkyl bromide was converted into the corresponding active Grignard reagent. With regard to the SmI_2 -mediated reaction, the initial RSmI_2 species (**A**, Figure 4.7) was readily formed as no starting alkyl halide was recovered. However, beside the desired cross-coupled product, side-products resulting from β -elimination, β -hydride transfer as well as homocoupling were observed by GC-MS.

According to the proposed mechanism, acceleration of the desired reaction by increasing the catalyst loading is not possible unless the excess of starting alkyl halide (RI, Figure 4.7) is also increased.³⁶ To start the catalytic cycle, part of species **A** has to form a complex (**B**) with CuBr and this amount is thereafter permanently tied up in the catalytic cycle. As a consequence, an amount of the starting alkyl halide RI equal to the catalyst loading is lost by definition. All things considered, both coupling procedures were abandoned as they consistently gave poor yields and were unlikely to perform at an acceptable level in equimolar cross-coupling reactions.



Figure 4.7 Proposed mechanism of the copper-catalyzed cross coupling of alkyl samarium reagents with alkyl halides.³⁶

4.6.2.2 Cross-couplings via formation of an olefin

As an alternative approach, we focused our attention on coupling procedures which generate a carbon-carbon double bond, which would then later be reduced to the saturated

system. In a comparative study, we evaluated the performance of the Wittig, the Horner-Wadsworth-Emmons (HWE) and the Julia-Kocienski³⁷ coupling for our system. The Julia-Kocienski coupling is a one-pot procedure (Figure 4.8) derived from the Julia-Lythgoe olefination,³⁸ which required a separate reductive elimination step.

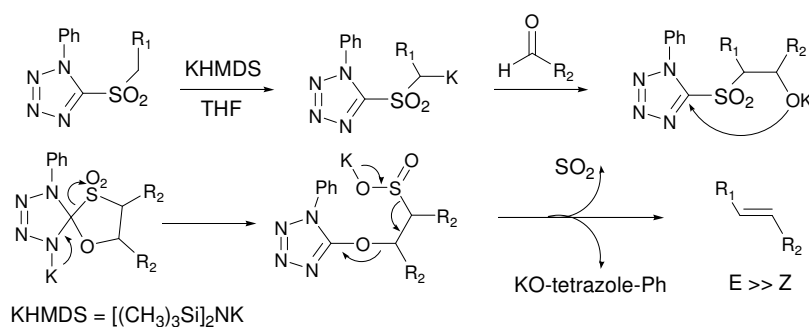
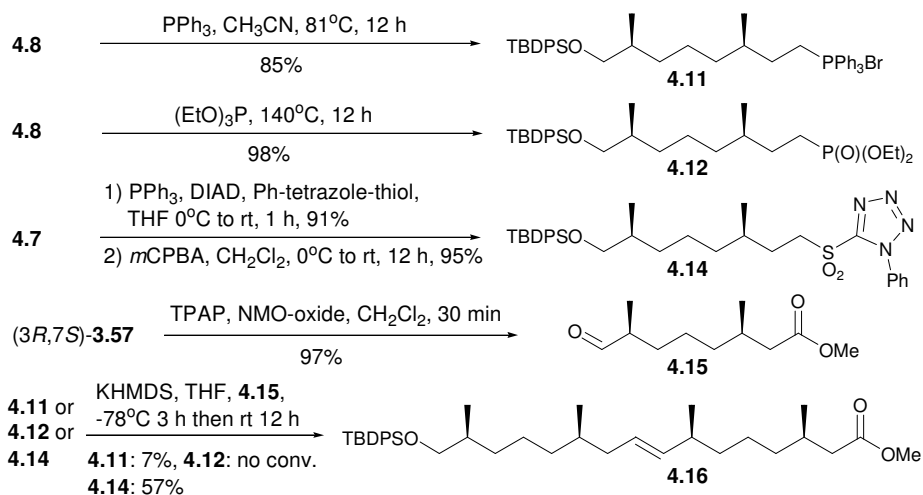


Figure 4.8 Mechanism of the Julia-Kocienski coupling.³⁷

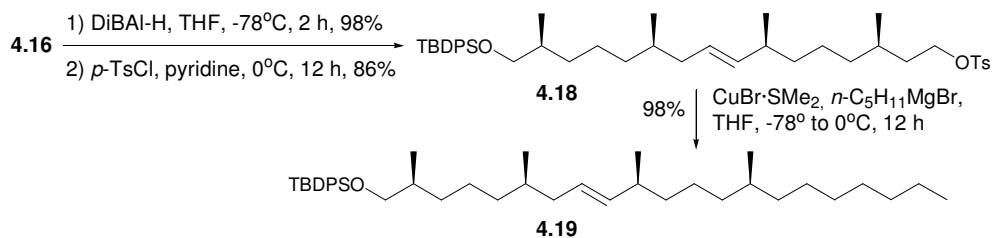
The Wittig reagent **4.11** was obtained in 85% yield from alkyl bromide **4.8** via reaction with PPh_3 (Scheme 4.3). Subjection of **4.8** to an Arbuzov reaction with triethylphosphite furnished HWE-reagent **4.12** (98%). Finally, the Julia-Kocienski sulfone was prepared from alcohol **4.7** via Mitsunobu reaction with 1-phenyl-1H-tetrazole-5-thiol (**4.13**, 91%) followed by oxidation of the resulting thioether with *meta*-chloroperoxybenzoic acid (*m*CPBA) to give **4.14** (95%).³⁹ Compounds **4.11**, **4.12** and **4.14** were subjected to coupling reactions with aldehyde **4.15**, which was obtained in 97% yield from (3*R*,7*S*)-**3.57** via oxidation with 4-methylmorpholine N-oxide (NMO-oxide) in the presence of a catalytic amount of tetra-*n*-propylammonium perruthenate (TPAP).⁴⁰ All three olefination reactions were performed in THF using a slight excess of **4.15** (1.1 eq) and potassium hexamethyldisilazane (KHMDS 1.0 eq) as the base. The Wittig reaction afforded diisoprenoid **4.16** in minute amounts (7%), while the desired product was not formed at all in the HWE-reaction. The Julia-Kocienski coupling gave the best result with an isolated yield of 57%. The reason why the Julia-Kocienski coupling performed better for this system than conventional Wittig- and HWE-reactions is unclear.

The next goal was to convert the methyl-ester of **4.16** into a terminal linear saturated C7 chain, for which we intended to employ the Grignard extension methodology already used in the synthesis of the apple leafminer pheromones (§ 3.4). In the event, the methyl ester

was reduced to the corresponding primary alcohol **4.17** (98%) with DiBAL-H, after which reaction with *p*-toluenesulfonyl chloride in pyridine afforded **4.18** in 86% yield (Scheme 4.4). Subsequent treatment with *n*-pentylmagnesium bromide and semi-catalytic CuBr·SMe₂ yielded **4.19** in 98%.

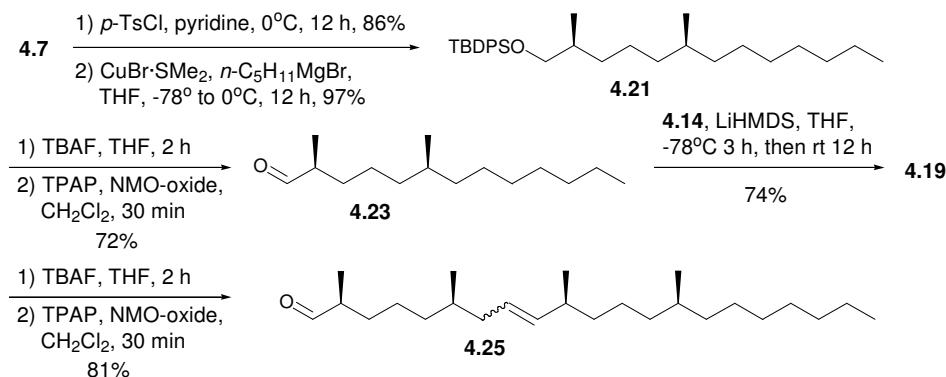


Scheme 4.3 Comparison of carbon-carbon double bond cross-coupling methods.



Scheme 4.4 Introduction of the saturated alkyl terminal chain.

Although an isolated yield of 57% in the preparation of diisoprenoid **4.16** was acceptable, we decided to investigate whether removal of the ester in **4.15** would be beneficial for the Julia-Kocienski coupling. To this end, an isoprenoid building block was elongated to obtain the terminal *n*-heptyl function prior to cross-coupling (Scheme 4.5). This slightly modified approach had the additional advantage that it made the route somewhat more convergent.



Scheme 4.5 Synthesis of diisoprenoid **4.25**.

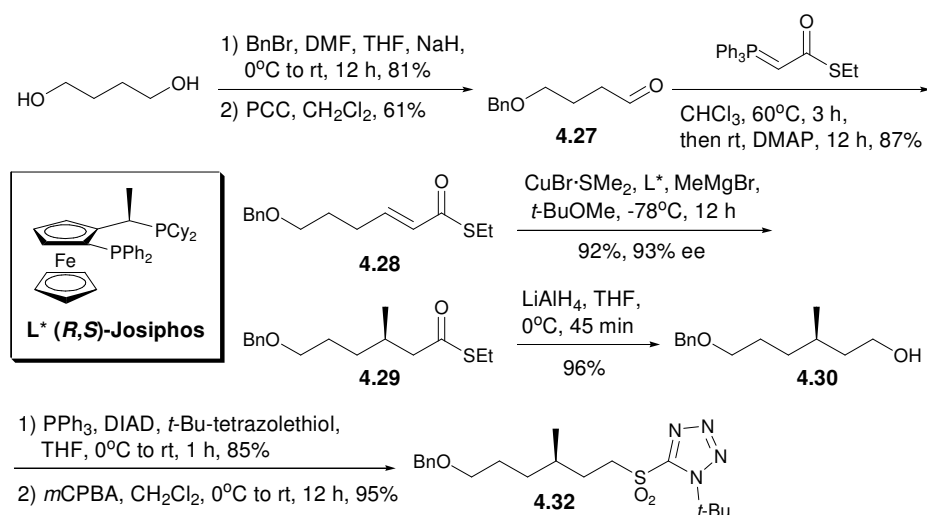
The conversion of **4.7** into the corresponding tosylate **4.20** (86%) and the subsequent coupling with *n*-pentylMgBr to obtain **4.21** (97%) was performed as described for the transformation of **4.17** into **4.19**. Treatment of **4.21** with TBAF yielded alcohol **4.22**, after which oxidation with NMO-oxide and TPAP furnished aldehyde **4.23** (72% over 2 steps). Julia-Kocienski coupling of **4.23** to sulfone **4.14** under comparable conditions as for the preparation of **4.16**, initially gave diisoprenoid **4.19** in similar yield (50-60%). However, changing the base from KHMDS to LiHMDS and mixing **4.23** and **4.14** prior to addition of the base (Barbier conditions) improved the yield to a gratifying 74%. It should be noted, that under these conditions, **4.19** was isolated as a mixture of *cis/trans* isomers (predominantly *trans*), whereas complete stereocontrol (only *trans*) was observed when KHMDS was used. Finally, the TBDPS-ether of **4.19** was cleaved and the resulting primary alcohol **4.24** was oxidized as before to give aldehyde **4.25** in 81% yield over two steps.

4.6.3 Completion of the oligoisoprenoid chain

As mentioned in paragraph 4.6.1, we intended to introduce the fifth methyl-branched stereogenic center (fragment **C**) using the asymmetric conjugate addition of Grignard reagents to α,β -unsaturated thioesters.³³

Synthesis of a suitable substrate for the key reaction started from 1,4-butanediol, which was mono-protected *via* reaction with benzyl bromide and sodium hydride to give **4.26** in 81% yield (Scheme 4.6). Even though the subsequent oxidation with pyridinium chlorochromate (PCC) proceeded quantitatively, the resulting aldehyde was isolated in a

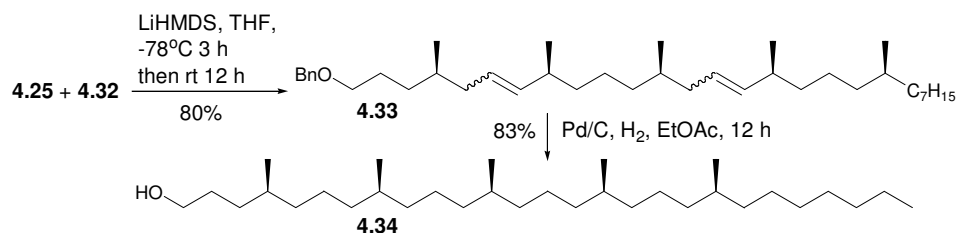
modest 61% yield due to the volatility of **4.27**. Finally, Wittig reaction of **4.27** with $\text{PPh}_3\text{CHCOSEt}$ afforded α,β -unsaturated thioester **4.28** as a mixture of *cis/trans* isomers.⁴¹ Stirring of the mixture in the presence of 4-dimethylaminopyridine (DMAP) shifted the equilibrium towards the desired *trans*-isomer, which was isolated in 87% yield after separation from remaining traces of the *cis*-isomer by column chromatography. Conjugate addition of MeMgBr to **4.28** in the presence of catalytic $\text{CuBr}\cdot\text{SMe}_2$ (5 mol%) and chiral ferrocene-based ligand L^* (6 mol%) resulted in the formation of (3*R*)-**4.29** (92%) with excellent stereoselectivity (93% ee).³³



Scheme 4.6 Synthesis of linear chiral fragment C.

In order to make fragment C suitable for cross-coupling, the thioester of **4.29** was reduced with LiAlH_4 to provide the corresponding primary alcohol **4.30** in 96% yield. Subsequent Mitsunobu reaction of **4.30** with 1-*tert*-butyltetrazole-5-thiol (**4.31**, 85%) followed by oxidation with *m*CPBA provided sulfone (3*R*)-**4.32** (95%). The rationale for the altered substitution moiety on the tetrazole ring in comparison to **4.14**, is that *tert*-butyltetrazolesulfones are known to often give superior yields, probably because of enhanced stability.⁴² In our case the improvement was not distinct, as LiHMDS -mediated Julia-Kocienski coupling of sulfone **4.32** to aldehyde **4.25** under Barbier conditions resulted in the formation of oligoisoprenoid **4.33** in 80% yield as a mixture of geometrical isomers (predominantly *trans*; Scheme 4.7). In comparison, coupling of **4.14** to **4.23** under identical

conditions gave **4.19** in 74% yield (Scheme 4.5). The synthesis of the oligoisoprenoid chain was completed by simultaneous deprotection of the primary alcohol and hydrogenation of the double bonds *via* exposure to hydrogen over Pd/C to give **4.34** in 83% yield. With the privilege of hindsight, a diimide reduction or hydrogenation over a different catalyst like, for example, Wilkinson catalyst ((PPh₃)₃RhCl) would have been a better choice to obtain the fully saturated system. Pd/C is known to occasionally cause migration of double bonds through reversible hydrometalation, which could lead to partial racemization of the stereogenic centers adjacent to the double bonds in our case.⁴³ Extensive evaluation of **4.34** by GC-analysis did not indicate that partial racemization/epimerization had indeed taken place.

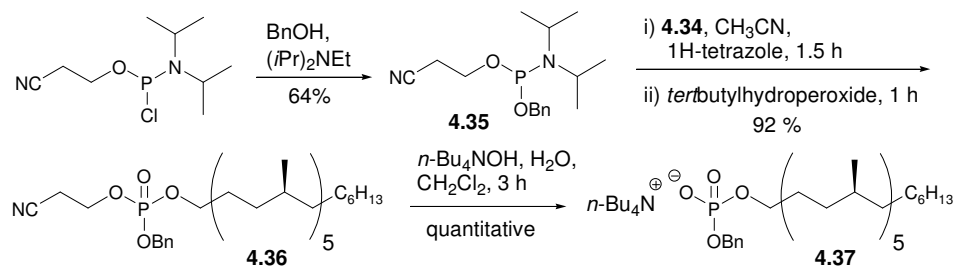


Scheme 4.7 Completion of the oligoisoprenoid chain **4.34**.

4.6.4 Formation of the β -D-mannosyl phosphate linkage

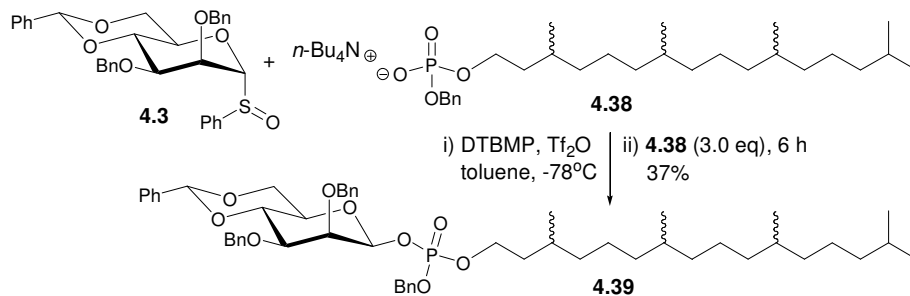
4.6.4.1 Modification of Crich's β -mannosylation protocol

Our first attempt to install the β -D-mannosyl phosphate linkage, was based on modification of Crich's β -mannosylation protocol (Scheme 4.1). We wondered how their approach would perform when a surplus of sulfoxide donor **4.3** was used rather than an excess of phosphate acceptor. To synthesize the appropriate phosphate acceptor, phosphoramidite **4.35** was prepared in 64% yield by reaction of benzyl alcohol with commercial 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite in the presence of Hunig's base (Scheme 4.8).² Subsequent tetrazole-mediated substitution of the diisopropylamine moiety of **4.35** by enantiopure saturated oligoisoprenoid **4.34** followed by oxidation of the resulting phosphite with *tert*-butyl hydroperoxide yielded phosphate ester **4.36** in 92%. Finally, treatment of **4.36** with tetra-*n*-butylammonium hydroxide in a dichloromethane/water biphasic system quantitatively afforded phosphate acceptor **4.37**.²

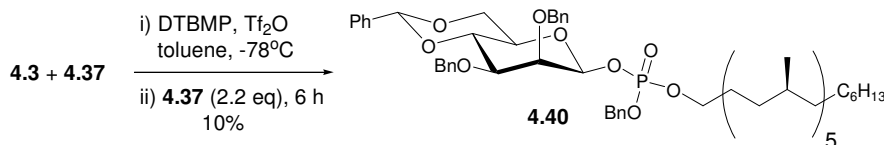


Scheme 4.8 Synthesis of tetra-*n*-butylammonium phosphate acceptor **4.37**.

To find the optimal coupling conditions, sulfoxide **4.3** was first connected to **4.38**, a racemic analogue of **4.37** which was synthesized from phytol (Scheme 4.9). Reproduction of Crich's procedure using three equivalents of **4.38** resulted in the isolation of **4.39** in 37% yield (literature^{30g}: 56%). The mass balance was made up mainly by the hydrolysis product of **4.3**, indicating that formation of the triflate was quantitative. When the amount of **4.38** was lowered to 1.5 equivalents, the yield of **4.39** dropped to 16%. Disappointingly, all reactions in which an excess of **4.3** was used failed to provide the desired product at all. The only attempt to couple **4.3** to enantiopure acceptor **4.37** (2.2 eq.) furnished the coupled product **4.40** in an unacceptable 10% yield (Scheme 4.10).



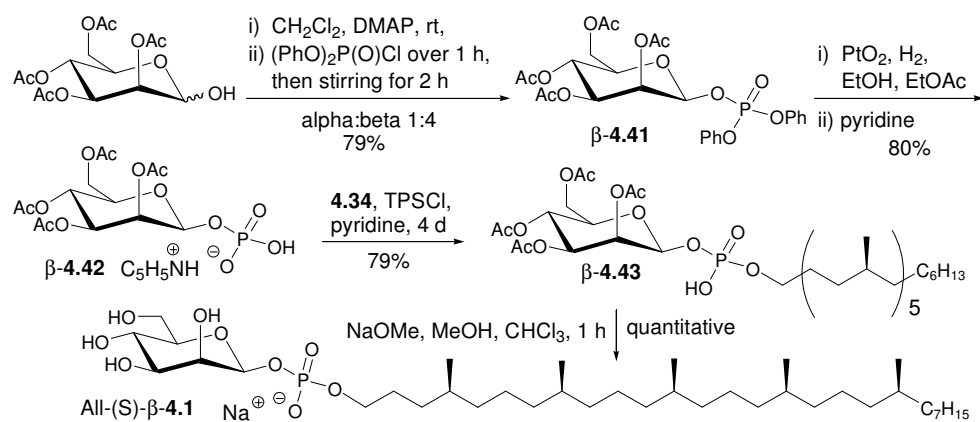
Scheme 4.9 Model system for the formation of the β -mannosyl phosphate linkage.



Scheme 4.10 Formation of the β -mannosyl phosphate linkage using Crich's protocol.

4.6.4.2 Reversed strategy; synthesis of a β-mannosyl phosphate ester and coupling to 4.34

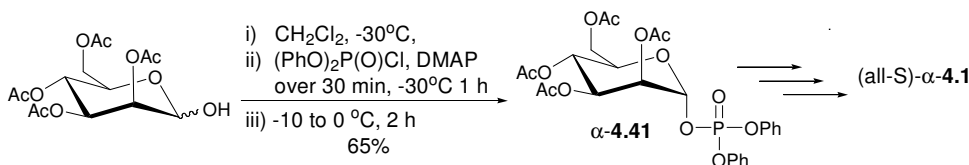
As an alternative approach, we decided to first install a β-phosphate moiety on a suitably protected mannopyranose, and then to connect the resulting β-mannosyl phosphate ester to the enantiopure oligoisoprenoid alcohol **4.34** (Scheme 4.11). To this end, diphenyl chlorophosphate was slowly added to a solution of 2,3,4,6-tetra-*O*-acetyl-D-mannose in the presence of excess DMAP at room temperature to give predominantly the β-anomer of mannosyl phosphate triester **4.41** (79%, α:β = 1:4).⁴⁴ The anomers were separated by column chromatography and the β-anomer proved to be stable in solution (CDCl₃) at room temperature. Remarkably, epimerization to the α-anomer did take place when **4.41** was concentrated, but this could be prevented by storage at -18 °C. Removal of the phenyl groups of β-**4.41** was achieved by hydrogenation over Adam's catalyst (PtO₂)⁴⁴ to give pyridinium salt β-**4.42** (80%) after quenching with pyridine. Subsequent coupling of an excess of readily available β-**4.42** (2.0 eq) to enantiopure **4.34** (1.0 eq) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl; 3.0 eq) proceeded readily to give **4.43** in a gratifying 79% yield after four days.⁴⁵ The employment of a sterically hindered arenesulfonyl chloride served to prevent sulfonylation of the primary alcohol of **4.34** which could otherwise compete with the desired phosphorylation reaction.⁴⁶ Finally, deacetylation with NaOMe in a mixture of chloroform and methanol afforded the target molecule (all-*S*)-β-**4.1** in quantitative yield (see § 4.6.6 for characterization).



Scheme 4.11 Completion of the synthesis; selective formation of a β-mannosyl phosphate ester followed by coupling to enantiopure alcohol **4.34** and deprotection.

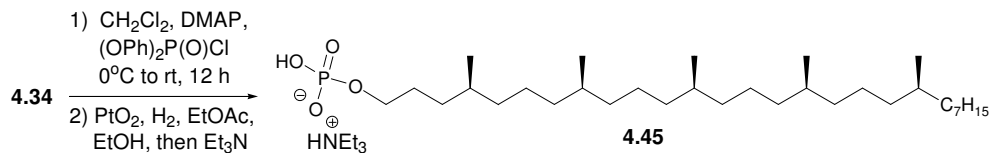
4.6.5 Synthesis of analogues

For analytical and biological comparison, the α -anomer of (all-S)-**4.1** was synthesized as well. In the event, α -**4.41** was selectively prepared in 65% isolated yield by simultaneous addition of diphenyl chlorophosphate and DMAP to a solution of 2,3,4,6-tetra-*O*-acetyl-D-mannose at -30 °C (Scheme 4.12).⁴⁴ The origin of this remarkable change in the stereochemical outcome of the phosphorylation reaction by a slight modification of the reaction conditions is related to the equilibrium between the two anomers of the starting material and their difference in reactivity. Due to the anomeric effect as well as the axial substituent at the neighboring carbon, the starting mannosylpyranose exists for over 85% as the α -anomer.⁴⁴ However, the same effects that cause instability of the β -anomer are also grounds for increased reactivity.⁴⁷ Hence, reaction in the presence of excess base (DMAP), which catalyzes the anomerization of the α - to the less stable β -form, mainly gives the kinetic β -product at room temperature. In contrast, simultaneous addition of the base and the phosphorylating agent results in the formation of exclusively the thermodynamic α -product at -30 °C. Conversion of α -**4.41** into (all-S)- α -**4.1** was performed in 49% overall yield following the same route as for the preparation of (all-S)- β -**4.1**.



Scheme 4.12 Synthesis of the α -mannosyl phosphomycoketide analogue.

To determine whether the (all-S)-phosphomycoketide, without the mannose headgroup, would show any biological activity, this compound was synthesized in two steps from enantiopure alcohol **4.34**. DMAP-assisted phosphorylation of **4.34** provided diphenyl phosphate triester **4.44**, after which hydrogenation over Adam's catalyst followed by quenching with triethylamine gave phosphate salt **4.45** in quantitative yield (Scheme 4.13).



Scheme 4.13 Synthesis of a phosphomycoketide analogue.

4.6.6 Proof of the anomeric configuration

Proof of the anomeric configuration of (all-S)- β -**4.1** commenced with a mass spectrometric investigation employing the same technique as was used in the structural elucidation of the natural product. Indeed, low-energy collisionally induced dissociation tandem mass spectrometry (ESI-CID-MS/MS) of (all-S)- β -**4.1** nicely reproduced the spectrum of the natural product (Figure 4.9).¹ Thus, a cross-ring fragmentation (m/z 587.7) of the carbohydrate moiety and a dehydration fragment (m/z 689.5) which are both characteristic for *cis*-1,2-glycosyl phosphates were observed.⁴⁸ In contrast, (all-S)- α -**4.1** produced a clearly different spectrum with the main fragment (m/z 545.5) originating from cleavage of the phosphomycoketide chain at the anomeric center.

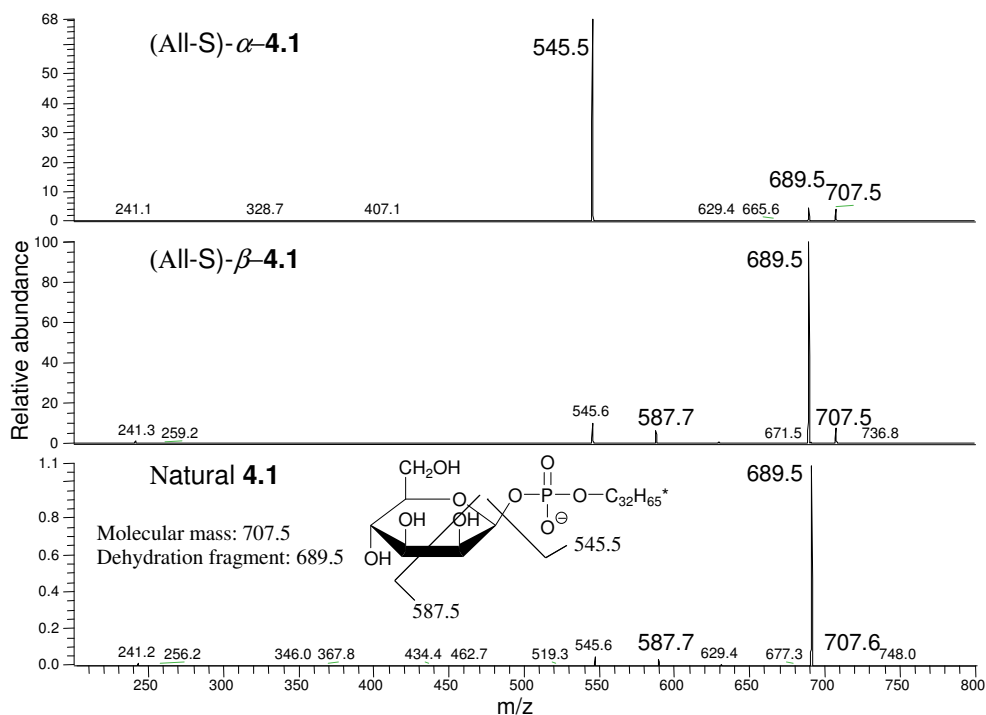


Figure 4.9 Low energy CID-MS/MS spectra of α - and β -(all-S)-**4.1** and natural **4.1**.¹

The anomeric configuration of (all-S)- β -**4.1** was confirmed by the NOE correlation between the anomeric hydrogen and H₃ and H₅ of the mannose moiety (1,3-diaxial interactions). Further evidence was obtained by comparison of the anomeric $^1J_{CH}$ coupling

constants, which as predicted by literature,⁴⁹ amounted to 169 Hz for the α -anomer and 159 Hz for the β -anomer. A final clue came from the ^1H -NMR spectra, which showed a clear difference in the chemical shift of the alpha- ($\delta = 5.38$ ppm) and the beta ($\delta = 5.05$ ppm) anomeric hydrogen (Figure 4.10; anomeric hydrogens depicted in inset).

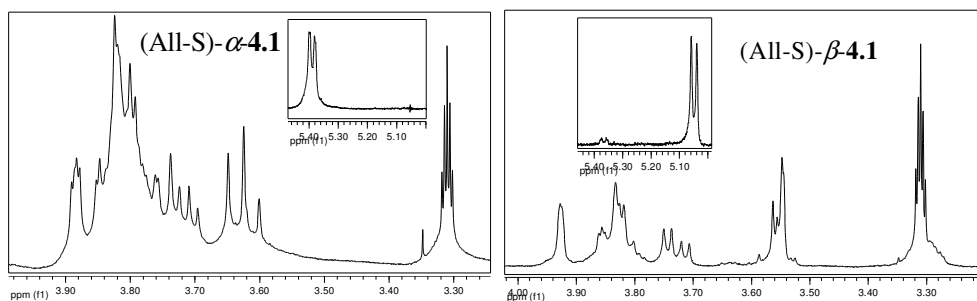


Figure 4.10 Comparison of part of the ^1H -NMR spectra of the two anomers of (all-S)-**4.1**; insert: anomeric protons.

4.7 Biological activity

In order to evaluate the biological activity of (all-S)- β -**4.1**, (all-S)- α -**4.1** and (all-S)-**4.45**, the compounds were tested in a dose-dependent manner in T-cell proliferation assays by Dr. Moody and co-workers.⁵⁰ CD8-1 T-cells ($\text{CD8}^+\text{TCR}$) were cultured with CD1c⁺ antigen presenting cells in the presence or absence of synthetic antigen. As an index of antigen-specific activation, interleukin-2 production was measured, which is secreted by CD8-1 T-cells to stimulate (among others) the proliferation of type 2 helper T-cells (HT-2 cells). In practice, an aliquot of the supernatant was removed from the bio-assay and diluted, after which HT-2 cells were cultured in this medium in the presence of [^3H]thymidine. The amount of [^3H]thymidine incorporated in DNA was measured with a liquid scintillation counter to give the Interleukin-2 production in counts per minute (CPM).

In agreement with the tri-molecular CD1-lipid-TCR model (§ 4.2.2), phosphomycoketide **4.45**, without the mannose moiety, did not show any activity unless elevated concentrations were used ($> 10 \mu\text{M}$) in which case activity might be caused by *in situ* mannosylation (Figure 4.11). In addition, comparison of (all-S)- β -**4.1** and (all-S)- α -**4.1** showed, that a relatively small structural change in the polar epitope already results in a significant decrease in activity. The most important finding, though, was that (all-S)- β -**4.1**

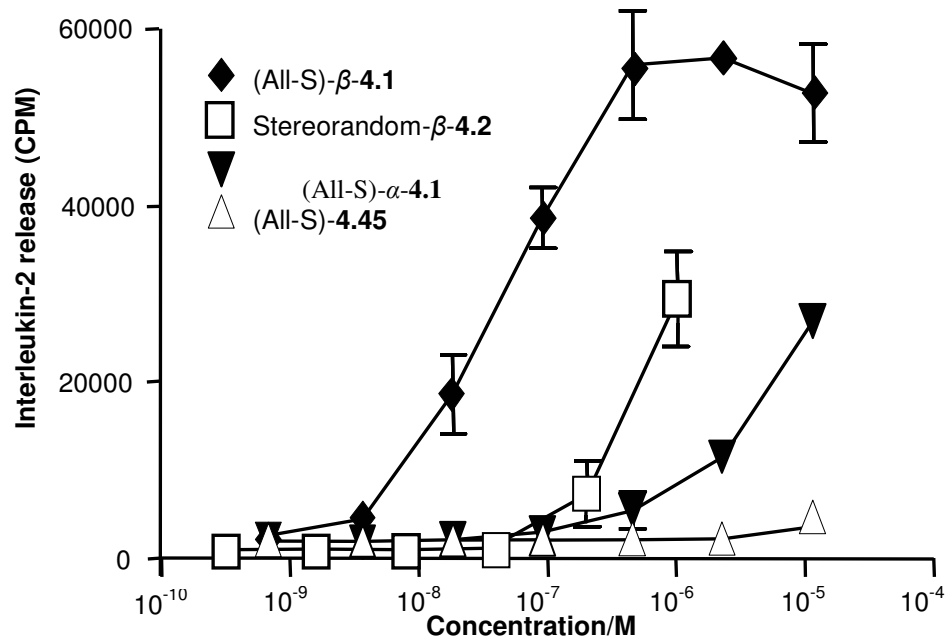


Figure 4.11 Biological activity of different synthetic MPM's.

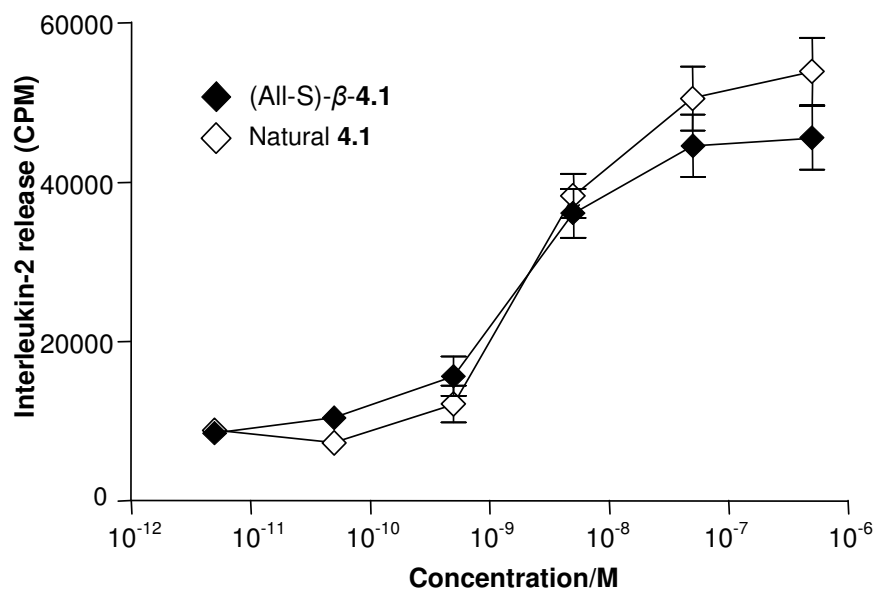


Figure 4.12 Biological activity of synthetic (all-S)- β -4.1 compared to the natural product.

has a considerably higher activity than stereorandom- β -**4.2**. It appears that, contrary to what was assumed so far, (the stereochemistry of) the oligoisoprenoid side-chain does have a pronounced effect on the activity. Finally, assessment of (all-S)- β -**4.1** in comparison to the natural product demonstrated that both compounds have identical activity within the margin of error (Figure 4.12). Obviously, this is an indication that the stereochemistry of the natural product could very well be all-S, although it is certainly no definite proof.

4.8 Summary and concluding remarks

In this chapter the first total synthesis of an enantiopure β -mannosyl phosphomycoketide (MPM) was described. To this end, a general and fully catalytic method for the preparation of enantiopure, saturated oligoisoprenoids of any desired length and stereochemistry was developed. In the event, two different conjugate addition reactions were employed to introduce the stereogenic centers with complete control over the absolute configuration, after which a Julia-Kocienski sequence was used to cross-couple the various linear enantiopure fragments. Using this strategy, penta-methyl-branched aliphatic alcohol **4.34** was prepared, with all stereogenic centers having the S-configuration. Low yields at the final stage of the synthesis due to the difficult nature of the β -mannosyl phosphate linkage were circumvented by installation of the phosphate moiety on a suitably protected mannopyranose prior to coupling to enantiopure **4.34**. Using this convergent approach, the total synthesis of (all-S)- β -**4.1** was achieved in 6.7% overall yield and a longest linear sequence of eighteen steps. In addition, the analogues (all-S)- α -**4.1** as well as (all-S)-**4.45**, which lacks the mannose unit, were prepared following similar routes.

Biological evaluation revealed that (all-S)- β -**4.1** and the natural product have identical activity, whereas an analogue with an oligoisoprenoid chain consisting of a mixture of 32 stereoisomers is significantly less active. The latter result implicates that the fine structure of the saturated alkyl chain influences biological activity to a far greater extent than assumed until now. From a biological point of view, *in vitro* and *in vivo* testing of a library of MPM's with variations in both the length and the stereochemistry of the oligoisoprenoid chain would be very helpful in acquiring detailed knowledge regarding the relationship between structure and activity. Since the synthesis of such a library is a laborious process, an X-ray structure of a CD1c-protein complexed to a β -mannosyl phosphomycoketide

antigen would be useful to set certain initial boundaries. At present, attempts are being made to co-crystallize a CD1c-protein and a molecule of synthetic (all-S)-**β-4.1**. If successful, the next objective would be to include a T-cell-receptor as well, which would provide the first direct structural evidence for the existence of the trimolecular complex (Figure 4.3).

4.9 Experimental section

For general information: see Chapter 2 and 3. HPLC analysis to determine the enantiomeric excess of **4.29** was carried out on a Shimadzu LC-10ADVP HPLC equipped with a Shimadzu SPD-M10AVP diode array detector. Racemic **4.29** was prepared by reaction of **4.28** with MeMgBr at -78°C in THF in the presence of CuI and TMSCl.

Phenyl-2,3-Di-O-benzyl-4,6-O-benzylidene-1-deoxy-1-thio-α-D-mannopyranoside S-oxide (4.3):^{30f,51c} Sulfoxide donor **4.3** was prepared in six steps from mannose using literature procedures.^{30f,51} NMR-data were as reported in the literature.^{30f,51c} ¹H-NMR (CDCl₃, 400 MHz) δ = 3.77 (t, *J* = 10.0 Hz, 1H), 4.12 (m, 1H), 4.23 (dd, *J* = 4.8, 10.4 Hz, 1H), 4.28-4.41 (m, 3H), 4.52 (brs, 1H), 4.57 (d, *J* = 11.6 Hz, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.84 (d, *J* = 12.0 Hz, 1H), 5.65 (s, 1H), 7.21-7.43 (m, 15H), 7.49-7.58 (m, 5H) ppm. MS(CI) for C₃₃H₃₂O₆S: *m/z* = 574 (M + NH₄)⁺.

8-(tert-Butyl-diphenyl-silanyloxy)-(3R,7S)-3,7-dimethyl-octanoic acid methyl ester (4.6):³⁵ (3R,7S)-**3.57** (1.62 g, 8.01 mmol) was dissolved in DMF (12.4 mL) and imidazole (1.09 g, 16.0 mmol, 2.0 eq) and TBDPSCl (4.15 mL, 16.0 mmol, 2.0 eq) were added. The resulting solution was stirred for 30 min under argon at room temperature, after which TLC showed complete conversion. The solution was diluted with water, extracted with Et₂O (3x) and the combined organic layers were washed with brine (sat.), dried (Na₂SO₄) and concentrated. **4.6** (3.34 g, 7.58 mmol, 95%) was isolated as a colorless oil after column chromatography (*n*-pentane-Et₂O 9:1). (3R,7S)-**4.6**: [α]_D²² = +1.7° (*c* = 1.97, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.91 (d, *J* = 6.4 Hz, 6H), 1.05 (s, 9H), 1.06-1.45 (m, 6H), 1.63 (m, 1H), 1.93 (m, 1H), 2.09 (dd, *J* = 8.4, 14.8 Hz, 1H), 2.29 (dd, *J* = 6.0, 14.8 Hz, 1H), 3.43 (dd, *J* = 6.4, 9.6 Hz, 1H), 3.50 (dd, *J* = 5.6, 9.6 Hz, 1H), 3.66 (s, 3H), 7.35-7.44 (m, 6H), 7.65-7.68 (m, 4H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 16.8 (q), 19.1 (s), 19.6 (q), 24.1 (t), 26.8 (q), 30.2 (d), 33.1 (t), 35.5 (d), 36.9 (t), 41.5 (t), 51.2 (q), 68.7 (t), 127.4 (d), 129.4 (d), 134.0 (s), 135.5 (d), 173.5 (s) ppm. MS(CI) for C₂₇H₄₀O₃Si: *m/z* = 458 (M + NH₄)⁺, HRMS calcd for C₂₇H₄₀O₃Si-C₄H₉: 425.251, found: 425.252.

8-(tert-Butyl-diphenyl-silanyloxy)-(3R,7S)-3,7-dimethyl-octan-1-ol (4.7):³⁵ To a solution of **4.6** (870 mg, 1.97 mmol) in THF (8.5 mL) at -78 °C was added a solution of Dibal-H

(20 wt% in toluene, 8.3 mL, 10.1 mmol, 5.0 eq) and the resulting mixture was stirred for 2 h at -78 °C under argon, after which TLC showed complete conversion.

The reaction was quenched with aq. NH₄Cl (sat.) and then diluted with Et₂O and aq. HCl (1.0 M) until a clear solution was obtained. The aqueous layer was extracted with Et₂O (3x) and the combined organic layers were washed with aq. NaHCO₃ (sat.) and brine (sat.), dried (Na₂SO₄) and concentrated. The product **4.7**, 792 mg, 1.92 mmol, 97%) was isolated as a colorless oil after column chromatography (*n*-pentane-EtOAc 4:1). (3R,7S)-**4.7**: [α]_D²² = +0.5° (c = 2.00, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.88 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H), 1.05 (s, 9H), 1.06-1.70 (m, 10H), 3.43 (dd, *J* = 6.4, 9.6 Hz, 1H), 3.51 (dd, *J* = 5.6, 9.6 Hz, 1H), 3.67 (m, 2H), 7.35-7.44 (m, 6H), 7.65-7.68 (m, 4H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 16.8 (q), 19.2 (s), 19.5 (q), 24.1 (t), 26.7 (q), 29.3 (d), 33.2 (t), 35.5 (d), 37.3 (t), 39.7 (t), 61.0 (t), 68.7 (t), 127.4 (d), 129.3 (d), 133.9 (s), 135.5 (d) ppm. MS(Cl) for C₂₆H₄₀O₂Si: *m/z* = 430 (M + NH₄)⁺, HRMS calcd for C₂₆H₄₀O₂Si-C₄H₉: 355.209, found: 355.207.

(8-Bromo-(2S,6R)-2,6-dimethyl-octyloxy)-tert-butyl-diphenyl-silane (4.8):³⁵ To a solution of **4.7** (188 mg, 0.46 mmol) in dichloromethane

(1.6 mL) were added PPh₃ (143 mg, 0.55 mmol, 1.2 eq) and then NBS (97 mg, 0.55 mmol, 1.2 eq) at 0 °C. The

resulting solution was stirred under argon at 0 °C for 10 min and then warmed to room temperature over 1 h. The reaction mixture was quenched with aq. NaHCO₃ (sat.) and the aqueous layer was extracted with dichloromethane (3x). The combined organic layers were washed with aq. Na₂S₂O₃ (10% w/w) and brine (sat.), dried (Na₂SO₄), filtered and concentrated. The resulting brown solid was suspended in *n*-pentane and filtered, after which the residue was washed with *n*-pentane. The filtrate was concentrated and further purified by column chromatography (*n*-pentane-Et₂O 99:1) to give **4.8** (191 mg, 0.40 mmol, 87%) as a colorless oil. (2S,6R)-**4.8**: [α]_D²² = -4.1° (c = 1.36, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.93 (d, *J* = 6.4 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 1.14 (s, 9H), 1.10-1.54 (m, 7H), 1.70 (m, 2H), 1.92 (m, 1H), 3.41-3.53 (m, 2H), 3.52 (dd, *J* = 6.4, 10.0 Hz, 1H), 3.59 (dd, *J* = 5.6, 9.6 Hz, 1H), 7.40-7.50 (m, 6H), 7.72-7.77 (m, 4H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 16.9 (q), 18.8 (q), 19.2 (s), 24.0 (t), 26.8 (q), 31.5 (d), 32.0 (t), 33.2 (t), 35.5 (d), 36.6 (t), 39.9 (t), 68.7 (t), 127.4 (d), 129.4 (d), 133.9 (s), 135.5 (d) ppm. MS(Cl) for C₂₆H₃₉BrOSi: *m/z* = 494 (M + NH₄)⁺. HRMS calcd for C₂₆H₃₉BrOSi-C₄H₉: 417.125, found: 417.126.

tert-Butyl-(8-iodo-(2S,6R)-2,6-dimethyl-octyloxy)-diphenyl-silane (4.9):³⁵ To a solution of PPh₃ (272 mg, 1.04 mmol, 1.2 eq) in dichloromethane

(2.5 mL) were added imidazole (70 mg, 1.04 mmol, 1.2 eq) and iodine (262 mg, 1.04 mmol, 1.2 eq) and the resulting

mixture was stirred under argon for 5 min. Subsequently, a solution of **4.7** (356 mg, 0.86 mmol) in dichloromethane (1.2 mL) was added and the mixture was stirred for 2 h, after which the reaction was quenched with aq. NaHCO₃ (sat.). The aqueous layer was extracted with dichloromethane (3x) and the combined organic layers were washed with aq. Na₂S₂O₃

(10% w/w) and brine (sat.), dried (Na₂SO₄), filtered and concentrated. The resulting white solid was suspended in *n*-pentane and filtered, after which the residue was washed with *n*-pentane. The filtrate was concentrated and further purified by column chromatography (*n*-pentane-Et₂O 99:1 to 98:2) to give **4.9** (418 mg, 0.80 mmol, 93%) as a colorless oil. ¹H-NMR (CDCl₃, 400 MHz) δ = 0.86 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H), 1.06 (s, 9H), 1.06-1.67 (m, 9H), 1.86 (m, 1H), 3.17 (m, 1H), 3.24 (m, 1H), 3.44 (dd, *J* = 6.0, 10.0 Hz, 1H), 3.51 (dd, *J* = 5.6, 10.0 Hz, 1H), 7.36-7.45 (m, 6H), 7.65-7.68 (m, 4H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 5.2 (t), 16.9 (q), 18.6 (q), 19.2 (s), 24.0 (t), 26.8 (q), 33.2 (t), 33.7 (d), 35.6 (d), 36.4 (t), 40.8 (t), 68.7 (t), 127.4 (d), 129.4 (d), 134.0 (s), 135.5 (d) ppm. MS(CI) for C₂₆H₃₉IOSi: *m/z* = 540 (M + NH₄)⁺.

8-Iodo-(3R,7S)-3,7-dimethyl-octanoic acid methyl ester (4.10):³⁵ To a solution of PPh₃ (207 mg, 0.79 mmol, 1.2 eq) in dichloromethane (1.9 mL) were added imidazole (54 mg, 0.79 mmol, 1.2 eq) and iodine (200 mg, 0.79 mmol, 1.2 eq) and the resulting mixture was stirred under argon for 5 min. Subsequently, a solution of (3R,7S)-**3.57** (133 mg, 0.66 mmol) in dichloromethane (0.94 mL) was added and the mixture was stirred for 2 h, after which the reaction was quenched with aq. NaHCO₃ (sat.). The aqueous layer was extracted with dichloromethane (3x) and the combined organic layers were washed with aq. Na₂S₂O₃ (10% w/w) and brine (sat.), dried (Na₂SO₄), filtered and concentrated. Purification by column chromatography (*n*-pentane-Et₂O 85:15) provided **4.10** (171 mg, 0.55 mmol, 83%) as a colorless liquid. ¹H-NMR (CDCl₃, 400 MHz) δ = 0.90 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 1.11-1.45 (m, 7H), 1.92 (m, 1H), 2.09 (dd, *J* = 8.0, 14.8 Hz, 1H), 2.26 (dd, *J* = 6.0, 14.8 Hz, 1H), 3.12 (dd, *J* = 5.6, 9.6 Hz, 1H), 3.19 (dd, *J* = 4.8, 9.6 Hz, 1H), 3.63 (s, 3H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 17.6 (t), 19.5 (q), 20.4 (q), 24.0 (t), 30.0 (d), 34.4 (d), 36.3 (t), 36.4 (t), 41.4 (t), 51.2 (q), 173.4 (s) ppm. MS(CI) for C₁₁H₂₁IO₂: *m/z* = 330 (M + NH₄)⁺.

[8-(tert-Butyl-diphenyl-silanyloxy)-(3R,7S)-3,7-dimethyl-octyl]-triphenyl-phosphonium; bromide (4.11): To a solution of **4.8** (197 mg, 0.41 mmol) in acetonitrile (4.0 mL) was added PPh₃ (130 mg, 0.50 mmol, 1.2 eq) and the resulting mixture was heated under reflux overnight. After cooling to room temperature, the reaction mixture was concentrated in vacuo. Purification by column chromatography (first *n*-pentane-Et₂O 98:2 to regain **4.8** and then dichloromethane-MeOH 95:5) furnished **4.11** (259 mg, 0.35 mmol, 85%) as a white solid. (3R,7S)-**4.11**: [α]_D²² = -4.4° (*c* = 1.13, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.82 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.99 (s, 9H), 1.00-1.42 (m, 7H), 1.55 (m, 2H), 1.68 (m, 1H), 3.34 (dd, *J* = 6.4, 10.0 Hz, 1H), 3.43 (dd, *J* = 6.0, 10.0 Hz, 1H), 3.59 (m, 2H), 7.28-7.36 (m, 6H), 7.58-7.68 (m, 10H), 7.72-7.81 (m, 9H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 16.6 (q), 18.9 (q), 19.0 (s), 20.4 + 20.9 (t, *J*_{C-P} = 50.7 Hz), 23.8 (t), 26.6 (q), 29.0 (t, *J*_{C-P} = 4.6 Hz), 33.0 (t), 33.3 (d), 35.4 (d), 36.5 (t), 68.6 (t), 117.5 + 118.4 (s, *J*_{C-P} = 85.9 Hz), 127.3 (d), 129.2 (d), 130.2 (d, *J* = 13.1 Hz), 133.4 (d, *J*_{C-P} = 10.0 Hz), 133.8 (s), 134.8 (d, *J* = 3.0 Hz), 135.3 (d) ppm.

[8-(*tert*-Butyl-diphenyl-silanyloxy)-(3*R*,7*S*)-3,7-dimethyl-octyl]-phosphonic acid diethyl ester (4.12):

A solution of **4.8** (197 mg, 0.41 mmol) in triethylphosphite (1.0 mL) was heated overnight at 140 °C while protected from moisture by a CaCl₂-tube. The reaction mixture was cooled to room temperature and then concentrated in vacuo. Purification by column chromatography (*n*-pentane-EtOAc 4:1 to 1:1) gave **4.12** (215 mg, 0.40 mmol, 98%) as a colorless oil. (3*R*,7*S*)-**4.12**: [α]_D²² = +1.7° (c = 1.39, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.84 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 7.2 Hz, 3H), 1.04 (s, 9H), 1.04-1.44 (m, 7H), 1.30 (t, *J* = 7.2 Hz, 6H), 1.53-1.80 (m, 3H), 3.42 (dd, *J* = 6.4, 10.0 Hz, 1H), 3.49 (dd, *J* = 6.0, 10.0 Hz, 1H), 4.00-4.15 (m, 6H), 7.30-7.42 (m, 6H), 7.63-7.67 (m, 4H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 16.3 (q, *J*_{C-P} = 6.1 Hz), 16.7 (q), 18.9 (q), 19.1 (s), 22.4 + 23.8 (t, *J*_{C-P} = 140 Hz), 24.1 (t), 26.7 (q), 28.9 (t, *J* = 5.3 Hz), 33.2 (t), 33.3 (d), 35.5 (d), 36.4 (t), 61.2 (t, *J*_{C-P} = 6.1 Hz), 68.7 (t), 127.4 (d), 129.3 (d), 133.9 (s), 135.4 (d) ppm. MS(EI) for C₃₀H₄₉O₄PSi-CH₃: *m/z* = 517 [M - CH₃]⁺. HRMS calcd for C₃₀H₄₉O₄PSi-CH₃: 517.290, found: 517.291.

5-[8-(*tert*-Butyl-diphenyl-silanyloxy)-(3*R*,7*S*)-3,7-dimethyl-octylthio]-1-phenyl-1H-tetrazole (4.13):

To a solution of **4.7** (450 mg, 1.09 mmol) and 1-phenyl-1H-tetrazole-5-thiol (389 mg, 2.18 mmol, 2.0 eq) in THF (10.0 mL) was added PPh₃ (430 mg, 1.64 mmol, 1.5 eq) at 0 °C.

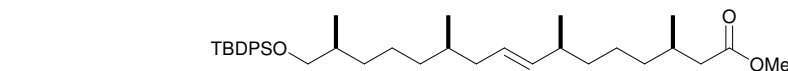
Subsequently, DIAD (0.38 mL, 1.9 mmol, 1.8 eq) was added dropwise over 2 min and the resulting solution was stirred under argon while warming to room temperature. After 1 h TLC showed complete conversion and the reaction was quenched with brine (sat.). The aqueous layer was extracted with Et₂O (3x) and the combined organic layers were dried (MgSO₄) and concentrated. Purification by column chromatography (*n*-pentane-EtOAc 9:1) gave **4.13** (571 mg, 1.00 mmol, 91%) as a colorless oil. (3*R*,7*S*)-**4.13**: [α]_D²² = -4.3° (c = 1.96, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.92 (dd, *J* = 5.6, 6.4 Hz, 6H), 1.06 (s, 9H), 1.06-1.70 (m, 9H), 1.81 (m, 1H), 3.34-3.54 (m, 4H), 7.36-7.44 (m, 6H), 7.51-7.60 (m, 5H), 7.67-7.69 (m, 4H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 16.8 (q), 19.0 (q), 19.2 (s), 24.0 (t), 26.7 (q), 31.2 (t), 32.0 (d), 33.2 (t), 35.5 (d), 35.8 (t), 36.7 (t), 68.7 (t), 123.7 (d), 127.4 (d), 129.3 (d), 129.6 (d), 129.9 (d), 133.6 (s), 133.9 (s), 135.5 (d), 154.3 (s) ppm. MS(CI) for C₃₃H₄₄N₄OSSi: *m/z* = 573 (M + H)⁺, 590 (M + NH₄)⁺, HRMS calcd for C₃₃H₄₄N₄OSSi-C₄H₉: 515.230, found: 515.230.

5-[8-(*tert*-Butyl-diphenyl-silanyloxy)-(3*R*,7*S*)-3,7-dimethyl-octane-1-sulfonyl]-1-phenyl-1H-tetrazole (4.14):

4.13 (570 mg, 0.99 mmol) was dissolved in dichloromethane (5.5 mL) and *m*CPBA (70%, 1.23 g, 4.97 mmol, 5.0 eq) was added at 0 °C. The resulting suspension was warmed to room temperature and stirred overnight. The reaction was quenched with aq. Na₂S₂O₃ (10% w/w) and diluted with dichloromethane. The organic layer was washed with aq. Na₂S₂O₃ (10% w/w, 3x; no more peroxides present) and the combined aqueous layers were subsequently extracted with dichloromethane (3x). The combined organic layers were washed with aq. NaHCO₃ (sat. 3x) and brine (sat.), dried (MgSO₄) and concentrated. The product was purified by column chromatography (*n*-pentane-EtOAc 9:1) to give **4.14** (575

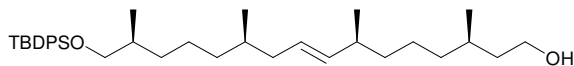
mg, 0.95 mmol, 95%) as a colorless oil. (3R,7S)-**4.14**: $[\alpha]_D^{22} = -4.1^\circ$ ($c = 1.74$, CHCl_3), $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) $\delta = 0.94$ (t, $J = 7.2$ Hz, 6H), 1.07 (s, 9H), 1.07-1.48 (m, 6H), 1.64 (m, 2H), 1.77 (m, 1H), 1.96 (m, 1H), 3.49 (m, 2H), 3.74 (m, 2H), 7.37-7.45 (m, 6H), 7.57-7.64 (m, 3H), 7.67-7.72 (m, 6H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100.6 MHz) $\delta = 16.7$ (q), 18.9 (q), 19.2 (s), 23.9 (t), 26.7 (q), 28.2 (t), 31.8 (d), 33.1 (t), 35.5 (d), 36.4 (t), 54.1 (t), 68.6 (t), 124.9 (d), 127.4 (d), 129.4 (d), 129.5 (d), 131.3 (d), 132.9 (s), 133.9 (s), 135.5 (d), 153.3 (s) ppm. MS(CI) for $\text{C}_{33}\text{H}_{44}\text{N}_4\text{O}_3\text{SSi}$: $m/z = 622$ ($\text{M} + \text{NH}_4$) $^+$, HRMS calcd for $\text{C}_{33}\text{H}_{44}\text{N}_4\text{O}_3\text{SSi}$ - C_4H_9 : 547.220, found: 547.223.

(3R,7S)-**3,7-Dimethyl-8-oxo-octanoic acid methyl ester (4.15)**: (3R,7S)-**3.57** (50 mg, 0.25 mmol) was dissolved in dichloromethane (2.3 mL) and NMO-oxide (43 mg, 0.37 mmol, 1.5 eq) and TPAP (17 mg, 49 μmol , 0.20 eq) were added. The resulting black solution was stirred for 30 min under argon, after which TLC showed conversion into a single product. The reaction mixture was brought directly onto a silica column and flushed with a mixture of *n*-pentane-EtOAc (95:5 to 9:1) to give pure **4.15** (48 mg, 0.24 mmol, 97%) as a colorless liquid after concentration. Because the product was particularly sensitive to oxidation, the pressure after concentration in vacuo was equalized with nitrogen instead of air and **6** was immediately used in the next step. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) $\delta = 0.91$ (d, $J = 6.4$ Hz, 3H), 1.08 (d, $J = 6.8$ Hz, 3H), 1.12-1.40 (m, 5H), 1.69 (m, 1H), 1.94 (m, 1H), 2.11 (dd, $J = 8.0, 14.8$ Hz, 1H), 2.28 (dd, $J = 6.0, 14.8$ Hz, 1H), 2.32 (m, 1H), 3.65 (s, 3H), 9.59 (d, $J = 2.0$ Hz, 1H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100.6 MHz) $\delta = 13.2$ (q), 19.5 (q), 24.1 (t), 30.0 (d), 30.4 (t), 36.4 (t), 41.4 (t), 46.1 (d), 51.3 (q), 173.5 (s), 205.1 (d) ppm.

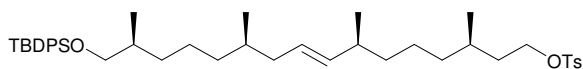


16-(tert-Butyl-diphenyl-silanyloxy)-(3R,7S,11R,15S)-3,7,11,15-tetramethyl-hexadec-8-enoic acid methyl ester (4.16): The experiment using sulfon **4.14** is described; procedures with Wittig reagent **4.11** and HWE-reagent **4.12** were performed in an analogous fashion. KHMDS (0.5 M in toluene, 0.69 mL, 0.35 mmol, 1.0 eq) was added in a dropwise fashion to a solution of **4.14** (196 mg, 0.32 mmol, 1.0 eq) in THF (4.0 mL) at -78°C resulting in a bright yellow solution. This mixture was stirred for 30 min under argon, after which **4.15** (73 mg, 0.36 mmol, 1.1 eq) in THF (4.0 mL) was added over 10 min. The solution was stirred for 3 h at -78°C and then stirred overnight while slowly warming to room temperature. The reaction was quenched with water and the aqueous layer was extracted with Et_2O (3x). The combined organic layers were washed with brine (sat.), dried (MgSO_4) and concentrated. Purification by column chromatography (*n*-pentane: Et_2O 98:2) gave **4.16** (106 mg, 0.18 mmol, 57%) as a colorless oil. In addition to **4.16**, several side products were observed, but due to the complexity of the mixture their structure could not be elucidated. (3R,7S,11R,15S)-**4.16**: $[\alpha]_D^{22} = +9.4^\circ$ ($c = 1.70$, CHCl_3), $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) $\delta = 0.83$ (d, $J = 6.8$ Hz, 3H), 0.92 (m, 6H), 0.95 (d, $J = 6.8$ Hz, 3H), 1.06 (s, 9H), 1.07-1.46 (m, 13H), 1.64 (m, 1H), 1.79 (m, 1H), 1.88-2.10 (m, 3H), 2.10 (dd, $J = 8.0, 14.4$ Hz, 1H), 2.30 (dd, $J = 6.0, 14.4$ Hz, 1H), 3.44 (dd, $J = 6.0, 10.0$ Hz, 1H), 3.52 (dd, $J = 6.0, 10.0$ Hz, 1H), 3.66 (s, 3H), 5.21 (dd, $J = 7.6, 15.2$ Hz, 1H), 5.31 (m, 1H), 7.36-7.44 (m, 6H), 7.66-7.69 (m, 4H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100.6 MHz) $\delta = 16.9$ (q), 19.2 (s), 19.3 (q), 19.6 (q), 21.0

(q), 24.3 (t), 24.5 (t), 26.8 (q), 30.2 (d), 33.1 (d), 33.3 (t), 35.6 (d), 36.6 (t), 36.6 (d), 36.7 (t), 37.0 (t), 39.8 (t), 41.6 (t), 51.2 (q), 68.8 (t), 126.9 (d), 127.4 (d), 129.3 (d), 134.0 (s), 135.5 (d), 137.4 (d), 173.7 (s) ppm. MS(CI) for $C_{37}H_{58}O_3Si$: $m/z = 597$ ($M + NH_4$)⁺, HRMS calcd for $C_{37}H_{58}O_3Si-C_4H_9$: 521.345, found: 521.346.

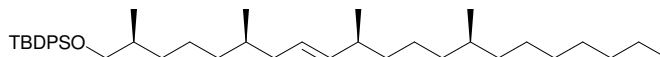


16-(tert-Butyl-diphenyl-silanyloxy)-(3R,7S,11R,15S)-3,7,11,15-tetramethyl-hexadec-8-en-1-ol (4.17): To a solution of **4.16** (100 mg, 0.173 mmol) in THF (6.0 mL) at $-78\text{ }^{\circ}\text{C}$ was added a solution of Dibal-H (20 wt% in toluene, 0.71 mL, 0.86 mmol, 5.0 eq) and the resulting mixture was stirred for 2 h at $-78\text{ }^{\circ}\text{C}$ under argon, after which TLC showed complete conversion. The reaction was quenched with aq. NH_4Cl (sat.) and then diluted with Et_2O and aq. HCl (1.0 M) until a clear solution was obtained. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were washed with aq. $NaHCO_3$ (sat.) and brine (sat.), dried (Na_2SO_4) and concentrated. The product (**4.17**, 93 mg, 0.169 mmol, 98%) was isolated as a colorless oil after column chromatography (*n*-pentane:EtOAc 4:1). (3R,7S,11R,15S)-**4.17**: $[\alpha]_D^{22} = +7.9^{\circ}$ ($c = 1.12$, $CHCl_3$), 1H -NMR ($CDCl_3$, 400 MHz) $\delta = 0.83$ (d, $J = 6.8$ Hz, 3H), 0.88 (d, $J = 6.8$ Hz, 3H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.95 (d, $J = 6.8$ Hz, 3H), 1.06 (s, 9H), 1.07-1.69 (m, 17H), 1.80 (m, 1H), 1.98 (m, 1H), 2.06 (m, 1H), 3.41 (dd, $J = 6.0, 10.0$ Hz, 1H), 3.51 (dd, $J = 6.0, 10.0$ Hz, 1H), 3.67 (m, 2H), 5.22 (dd, $J = 7.6, 15.2$ Hz, 1H), 5.32 (m, 1H), 7.36-7.44 (m, 6H), 7.66-7.68 (m, 4H) ppm. ^{13}C -NMR ($CDCl_3$, 100.6 MHz) $\delta = 16.9$ (q), 19.2 (s), 19.4 (q), 19.5 (q), 21.0 (q), 24.3 (t), 24.5 (t), 26.8 (q), 29.3 (d), 33.1 (d), 33.3 (t), 35.6 (d), 36.69 (t), 36.71 (d), 37.0 (t), 37.3 (t), 39.87 (t), 39.90 (t), 61.1 (t), 68.8 (t), 126.8 (d), 127.4 (d), 129.3 (d), 134.0 (s), 135.5 (d), 137.5 (d) ppm. MS(CI) for $C_{36}H_{58}O_2Si$: $m/z = 568$ ($M + NH_4$)⁺, HRMS calcd for $C_{36}H_{58}O_2Si-C_4H_9$: 493.350, found: 493.349.



16-(tert-Butyl-diphenyl-silanyloxy)-(3R,7S,11R,15S)-3,7,11,15-tetramethyl-hexadec-8-en-1-yl *p*-toluenesulfonate (4.18): **4.17** (90 mg, 0.16 mmol) was dissolved in dry pyridine (0.62 mL) and *p*-TsCl (47 mg, 0.25 mmol, 1.5 eq) was added at $0\text{ }^{\circ}\text{C}$. The resulting solution was stirred at $0\text{ }^{\circ}\text{C}$ under argon overnight and then quenched with water. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were washed with aq. $CuSO_4$ (sat.), aq. $NaHCO_3$ (sat.) and brine (sat.), dried ($MgSO_4$) and concentrated to give **4.18** (99 mg, 0.14 mmol, 86%) as a colorless oil after purification by column chromatography (*n*-pentane-EtOAc 95:5). (3R,7S,11R,15S)-**4.18**: $[\alpha]_D^{22} = +7.9^{\circ}$ ($c = 1.46$, $CHCl_3$), 1H -NMR ($CDCl_3$, 400 MHz) $\delta = 0.78$ (d, $J = 6.4$ Hz, 3H), 0.82 (d, $J = 6.4$ Hz, 3H), 0.92 (t, $J = 6.8$ Hz, 6H), 1.05 (s, 9H), 1.06-1.70 (m, 17H), 1.78 (m, 1H), 2.00 (m, 2H), 2.45 (s, 3H), 3.43 (dd, $J = 6.4, 9.6$ Hz, 1H), 3.51 (dd, $J = 5.2, 9.6$ Hz, 1H), 4.05 (m, 2H), 5.20 (dd, $J = 7.6, 15.2$ Hz, 1H), 5.30 (m, 1H), 7.33-7.44 (m, 8H), 7.66-7.68 (dd, $J = 1.6, 7.6$ Hz, 4H), 7.79 (d, $J = 8.4$ Hz, 2H) ppm. ^{13}C -NMR ($CDCl_3$, 100.6 MHz) $\delta = 16.9$ (q), 19.0 (q), 19.2 (s), 19.3 (q), 20.9 (q), 21.5 (q), 24.3 (t), 26.8 (q), 29.0 (d), 33.0 (d), 33.3 (t), 35.60 (d), 35.63 (t), 36.5 (t), 36.6 (d), 36.7 (t), 37.1 (t), 39.8 (t), 68.8 (t), 69.0 (t), 126.9 (d), 127.4 (d), 127.8 (d), 129.3 (d), 129.7 (d), 133.1 (s), 134.0 (s), 135.5 (d), 137.4 (d), 144.5 (s) ppm.

MS(Cl) for $C_{43}H_{64}O_4SSi$: $m/z = 722$ ($M + NH_4$)⁺, HRMS calcd for $C_{43}H_{64}O_4SSi-C_4H_9$: 647.359, found: 647.360.



tert-Butyl-diphenyl-((2S,6R,10S,14S)-2,6,10,14-tetramethyl-henicos-8-enyloxy)-silane (4.19): *n*-Pentyl bromide (0.50 mL, 4.0 mmol) in dry THF (9.5 mL) was added dropwise to Mg (147 mg, 6.05 mmol, 1.5 eq) in an argon-purged flask and then stirred for 1 h at 45 °C. The resulting solution was cooled to room temperature and the precipitate was allowed to settle overnight. Titration with *sec*-butanol in the presence of 1,10-phenanthroline showed the concentration of $C_5H_{11}MgBr$ to be 0.43 M. The Grignard reagent (1.72 mL, 0.740 mmol, 4.3 eq) was added in a dropwise fashion to a solution of **4.18** (120 mg, 0.171 mmol) and $CuBr \cdot SMe_2$ (9.5 mg, 46 μ mol, 27 mol%) in dry THF (1.6 mL) at -78 °C under argon. After stirring for 1 h at -78 °C, the solution was allowed to warm to 0 °C and stirred overnight. The reaction was quenched with aq. NH_4Cl (sat.), extracted with Et_2O (3x) and the combined organic layers were washed with brine (sat.), dried ($MgSO_4$) and concentrated. **4.19** (101 mg, 0.167 mmol, 98%) was isolated as a colorless oil after purification by column chromatography (*n*-pentane). (2S,6R,10S,14S)-**4.19**: $[\alpha]_D^{22} = +6.0^\circ$ ($c = 1.51$, $CHCl_3$), 1H -NMR ($CDCl_3$, 400 MHz) $\delta = 0.82$ -0.97 (m, 15H), 1.06 (s, 9H), 1.07 (m, 4H), 1.14-1.46 (m, 22H), 1.65 (m, 1H), 1.80 (m, 1H), 1.94-2.12 (m, 2H), 3.43 (dd, $J = 6.0$, 10.0 Hz, 1H), 3.51 (dd, $J = 5.6$, 10.0 Hz, 1H), 5.20-5.36 (m, 2H), 7.36-7.45 (m, 6H), 7.66-7.70 (m, 4H) ppm. ^{13}C -NMR ($CDCl_3$, 100.6 MHz) $\delta = 14.0$ (q), 16.9 (q), 19.2 (s), 19.4 (q), 19.6 (q), 21.0 (q), 22.6 (t), 24.3 (t), 24.7 (t), 26.8 (q), 27.0 (t), 29.3 (t), 29.9 (t), 31.8 (t), 32.6 (d), 33.1 (d), 33.3 (t), 35.6 (d), 36.70 (t), 36.72 (d), 37.0 (t), 37.4 (t), 39.9 (t), 68.8 (t), 126.7 (d), 127.4 (d), 129.3 (d), 134.0 (s), 135.5 (d), 137.7 (d) ppm. MS(Cl) for $C_{41}H_{68}OSi$: $m/z = 623$ ($M + NH_4$)⁺, HRMS calcd for $C_{41}H_{68}OSi-C_4H_9$: 547.434, found: 547.434.

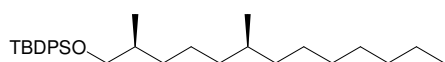
tert-Butyl-diphenyl-((2S,6R,10S,14S)-2,6,10,14-tetramethyl-henicos-8-enyloxy)-silane (4.19) from 4.14 and 4.23: To a solution of **4.14** (964 mg, 1.59 mmol) and **4.23** (415 mg, 1.83 mmol, 1.15 eq) in THF (19 mL) at -78 °C was added LiHMDS (1.0 M in THF, 1.67 mL, 1.67 mmol, 1.05 eq) in a dropwise fashion over 5 min. The resulting yellow solution was stirred at -78 °C for 3 h and then allowed to warm to room temperature overnight. The reaction was quenched with aq. NH_4Cl (sat.) and the aqueous layer was extracted with Et_2O (3x). The combined organic layers were washed with brine (sat.), dried ($MgSO_4$) and concentrated. Purification by column chromatography (*n*-pentane) gave **4.19** (713 mg, 1.18 mmol, 74%) as a colorless oil. Analytical data are shown above.

8-(tert-Butyl-diphenyl-silanyloxy)-(3R,7S)-3,7-dimethyl-octyl *p*-toluenesulfonate

(4.20): **4.7** (1.96 g, 4.76 mmol) was dissolved in dry pyridine (17.7 mL) and *p*-TsCl (1.36 g, 7.13 mmol, 1.5 eq) was added at 0 °C. The resulting solution was stirred at 0 °C under argon for 36 h and then quenched with water. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were washed with aq. $CuSO_4$ (sat. 2x), aq. $NaHCO_3$ (sat.) and brine (sat.), dried ($MgSO_4$) and concentrated to give **4.20** (2.33 g, 4.11 mmol, 86%) as a colorless oil after purification by column chromatography (*n*-pentane-EtOAc 95:5). (3R,7S)-**4.20**: $[\alpha]_D^{22} = -1.0^\circ$ ($c = 1.27$, $CHCl_3$), 1H -NMR ($CDCl_3$,

400 MHz) δ = 0.79 (d, J = 6.4 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H), 1.06 (s, 9H), 1.06-1.52 (m, 8H), 1.64 (m, 2H) 2.44 (s, 3H), 3.43 (dd, J = 6.4, 10.0 Hz, 1H), 3.50 (dd, J = 5.6, 10.0 Hz, 1H), 4.06 (m, 2H), 7.32-7.44 (m, 8H), 7.66-7.68 (m, 4H), 7.80 (d, J = 8.4 Hz, 2H) ppm. ^{13}C -NMR (CDCl_3 , 100.6 MHz) δ = 16.8 (q), 19.0 (q), 19.2 (s), 21.5 (q), 24.0 (t), 26.8 (q), 29.1 (d), 33.2 (t), 35.5 (d), 35.6 (t), 36.8 (t), 68.7 (t) 69.0 (t), 127.4 (d), 127.8 (d), 129.4 (d), 129.7 (d), 133.1 (s), 134.0 (s), 135.5 (d), 144.5 (s) ppm. MS(CI) for $\text{C}_{33}\text{H}_{46}\text{O}_4\text{SSi}$: m/z = 584 ($\text{M} + \text{NH}_4$) $^+$, HRMS calcd for $\text{C}_{33}\text{H}_{46}\text{O}_4\text{SSi}-\text{C}_4\text{H}_9$: 509.218, found: 509.219.

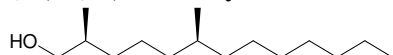
***tert*-Butyl-((2*S*,6*S*)-2,6-dimethyl-tridecyloxy)-diphenyl-silane (4.21):** Pentyl bromide



(2.50 mL, 20.2 mmol) in dry THF (47.5 mL) was added dropwise to Mg (735 mg, 30.2 mmol, 1.5 eq) in an argon-purged flask and then stirred

for 1 h at 45 °C. The resulting solution was cooled to room temperature and the precipitate was allowed to settle overnight. Titration with *sec*-butanol in the presence of 1,10-phenanthroline showed the concentration of $\text{C}_5\text{H}_{11}\text{MgBr}$ to be 0.39 M. The Grignard reagent (41.7 mL, 16.3 mmol, 4.0 eq) was added over 40 min to a solution of **4.20** (2.30 g, 4.06 mmol) and $\text{CuBr}\cdot\text{SMe}_2$ (209 mg, 1.02 mmol, 25 mol%) in dry THF (38 mL) at -78 °C under argon. After stirring for 2 h at -78 °C, the solution was allowed to warm to 0 °C and stirred overnight. The reaction was quenched with aq. NH_4Cl (sat.), extracted with Et_2O (3x) and the combined organic layers were washed with brine (sat.), dried (MgSO_4) and concentrated. **4.21** (1.84 g, 3.94 mmol, 97%) was isolated as a colorless oil after purification by column chromatography (*n*-pentane). (2*S*,6*S*)-**4.21**: $[\alpha]_{\text{D}}^{20}$ = -0.8° (c = 1.27, CHCl_3), ^1H -NMR (CDCl_3 , 400 MHz) δ = 0.86 (d, J = 6.8 Hz, 3H), 0.92 (t, J = 7.2 Hz, 3H), 0.96 (d, J = 6.8 Hz, 3H), 1.09 (s, 9H), 1.06-1.52 (m, 19H), 1.67 (m, 1H) 3.47 (dd, J = 6.4, 9.6 Hz, 1H), 3.50 (dd, J = 5.6, 9.6 Hz, 1H), 7.38-7.46 (m, 6H), 7.70-7.72 (m, 4H) ppm. ^{13}C -NMR (CDCl_3 , 100.6 MHz) δ = 14.0 (q), 16.9 (q), 19.2 (s), 19.6 (q), 22.6 (t), 24.3 (t), 26.8 (q), 27.0 (t), 29.3 (t), 29.9 (t), 31.9 (t), 32.6 (d), 33.4 (t), 35.6 (d), 37.0 (t), 37.3 (t), 68.8 (t), 127.4 (d), 129.4 (d), 134.1 (s), 135.5 (d) ppm. MS(CI) for $\text{C}_{31}\text{H}_{50}\text{OSi}$: m/z = 484 ($\text{M} + \text{NH}_4$) $^+$, HRMS calcd for $\text{C}_{31}\text{H}_{50}\text{OSi}-\text{C}_4\text{H}_9$: 409.293, found: 409.294.

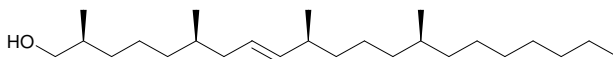
(2*S*,6*S*)-2,6-Dimethyl-tridecan-1-ol (4.22): TBAF (1.0 M in THF, 15.7 mL, 15.7 mmol,



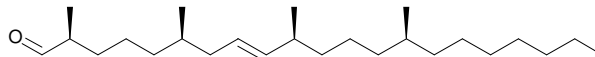
4.0 eq) was added to a solution of **4.21** (1.83 g, 3.92 mmol) in THF (26.5 mL) and the resulting mixture

was stirred for 2 h at room temperature, after which TLC showed complete conversion. The reaction was quenched with aq. NH_4Cl (sat.), extracted with Et_2O (3x) and the combined organic layers were washed with brine (sat.), dried (MgSO_4) and concentrated. Because **4.22** was difficult to separate from the TBDPS-waste products, the crude product was used in the next step. An analytical sample was purified by column chromatography (benzene: Et_2O 95:5 and then a second column using *n*-pentane- Et_2O 9:1 to 4:1) to give a colorless oil. (2*S*,6*S*)-**4.22**: $[\alpha]_{\text{D}}^{22}$ = -8.0° (c = 1.05, CHCl_3), ^1H -NMR (CDCl_3 , 400 MHz) δ = 0.84 (d, J = 6.4 Hz, 3H), 0.87 (t, J = 6.8 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 1.07 (m, 3H), 1.18-1.40 (m, 16H), 1.46 (brs, 1H), 1.61 (m, 1H), 3.40 (dd, J = 6.8, 10.4 Hz, 1H), 3.50 (dd, J = 5.6, 10.4 Hz, 1H) ppm. ^{13}C -NMR (CDCl_3 , 100.6 MHz) δ = 14.0 (q), 16.5 (q), 19.6 (q), 22.6 (t), 24.3 (t), 27.0 (t), 29.3 (t), 29.9 (t), 31.8 (t), 32.6 (d), 33.4 (t), 35.7 (d), 36.9 (t), 37.2 (t), 68.3 (t) ppm. MS(EI) for $\text{C}_{15}\text{H}_{32}\text{O}$: m/z = 210 [M^+], HRMS calcd for $\text{C}_{15}\text{H}_{32}\text{O}$: 210.235, found: 210.235.

(2S,6S)-2,6-Dimethyl-tridecanal (4.23): Crude **4.22** (3.92 mmol) was dissolved in dry dichloromethane (37 mL) in the presence of crushed molsieves (4Å) and stirred for 20 min. Subsequently, NMO-oxide (668 mg, 5.70 mmol, 1.50 eq) and TPAP (80 mg, 0.23 mmol, 0.06 eq) were added. The resulting black solution was stirred for 45 min, after which TLC showed conversion into a single product. The reaction mixture was filtered over a silica plug (dichloromethane) and concentrated. After column chromatography (*n*-pentane-EtO₂ 98:2) **4.23** (639 mg, 2.82 mmol, 72% from **4.21**) was obtained as a colorless liquid. Because the product was sensitive to oxidation, the pressure after concentration in vacuo was equalized with nitrogen instead of air and (2S,6S)-**4.23** was immediately used in the next step. ¹H-NMR (CDCl₃, 400 MHz) δ = 0.84 (d, *J* = 6.4 Hz, 3H), 0.88 (t, *J* = 6.8 Hz, 3H), 1.07 (m, 2H), 1.09 (d, *J* = 6.8 Hz, 3H), 1.18-1.40 (m, 16H), 1.69 (m, 1H), 2.34 (ddd, *J* = 2.0, 6.8, 13.6 Hz, 1H) ppm. ¹³C-NMR (CDCl₃, 50.32 MHz) δ = 13.3 (q), 14.0 (q), 19.5 (q), 22.6 (t), 24.3 (t), 26.9 (t), 29.3 (t), 29.8 (t), 30.8 (t), 31.8 (t), 32.5 (d), 33.8 (t), 36.9 (t), 46.3 (d), 205.2 (d) ppm. MS(CI) for C₁₅H₃₀O+O (oxidized to carboxylic acid): *m/z* = 260 (*M* + NH₄)⁺.



(2S,6R,10S,14S)-2,6,10,14-Tetramethyl-henicos-8-en-1-ol (4.24): TBAF (1.0 M in THF, 6.3 mL, 6.3 mmol, 4.0 eq) was added to a solution of **4.23** (958 mg, 1.58 mmol) in THF (10.6 mL) and the resulting mixture was stirred for 2 h, after which TLC showed complete conversion. The reaction was quenched with aq. NH₄Cl (sat.), extracted with Et₂O (3x) and the combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. Because **4.24** was difficult to separate from the TBDPS-waste products, the crude product was used in the next step. An analytical sample was purified by column chromatography (benzene-Et₂O 95:5) to give a colorless oil. (2S,6R,10S,14S)-**4.24**: [α]_D²² = +6.1° (*c* = 1.06, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.83 (d, *J* = 7.2 Hz, 3H), 0.84 (d, *J* = 7.2 Hz, 3H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 1.06 (m, 4H), 1.14-1.48 (m, 23H), 1.61 (m, 1H), 1.81 (m, 1H), 1.98 (m, 1H), 2.05 (m, 1H), 3.41 (dd, *J* = 6.4, 10.0 Hz, 1H), 3.51 (dd, *J* = 5.6, 10.0 Hz, 1H), 5.22 (dd, *J* = 7.2, 15.2 Hz, 1H), 5.31 (m, 1H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 14.0 (q), 16.5 (q), 19.4 (q), 19.6 (q), 21.0 (q), 22.6 (t), 24.3 (t), 24.7 (t), 27.0 (t), 29.3 (t), 29.9 (t), 31.8 (t), 32.7 (d), 33.1 (d), 33.3 (t), 35.7 (d), 36.65 (t), 36.72 (d), 37.0 (t), 37.4 (t), 39.9 (t), 68.3 (t), 126.6 (d), 137.8 (d) ppm. MS(EI) for C₂₅H₅₀O: *m/z* = 366 [*M*]⁺, HRMS calcd for C₂₅H₅₀O: 366.386, found: 366.385.



(2S,6R,10S,14S)-2,6,10,14-Tetramethyl-henicos-8-enal (4.25): Crude **4.24** (1.58 mmol) was dissolved in dry dichloromethane (15.4 mL) in the presence of crushed molsieves (4Å) and stirred for 20 min. Subsequently, NMO-oxide (278 mg, 2.37 mmol, 1.50 eq) and TPAP (33 mg, 0.10 mmol, 0.06 eq) were added. The resulting black solution was stirred for 30 min, after which TLC showed conversion into a single product. The reaction mixture was filtered over a silica plug (dichloromethane) and concentrated. After column chromatography (*n*-pentane-EtO₂ 98:2) **4.25** (468 mg, 1.28 mmol, 81% from **4.23**) was

obtained as a colorless oil. Because the product was sensitive to oxidation, the pressure after concentration in vacuo was equalized with nitrogen instead of air and **4.25** was immediately used in the next step. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ = 0.82 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 0.88 (t, J = 6.8 Hz, 3H), 0.95 (d, J = 6.4 Hz, 3H), 1.09 (d, J = 6.8 Hz, 3H), 1.09 (m, 4H), 1.16-1.50 (m, 21H), 1.69 (m, 1H), 1.82 (m, 1H), 1.97 (m, 1H), 2.05 (m, 1H), 2.33 (m, 1H), 5.20-5.34 (m, 2H), 9.61 (d, J = 2.0 Hz, 1H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100.6 MHz) δ = 13.2 (q), 14.0 (q), 19.3 (q), 19.6 (q), 21.0 (q), 22.6 (t), 24.3 (t), 24.6 (t), 27.0 (t), 29.3 (t), 29.9 (t), 30.7 (t), 31.8 (t), 32.6 (d), 32.9 (d), 36.3 (t), 36.7 (d), 37.0 (t), 37.4 (t), 39.8 (t), 46.2 (d), 126.4 (d), 137.9 (d), 205.3 (d) ppm. MS(EI) for $\text{C}_{25}\text{H}_{48}\text{O}$: m/z = 364 [M^+], HRMS calcd for $\text{C}_{25}\text{H}_{48}\text{O}$: 364.371, found: 364.373.

4-Benzyloxy-butan-1-ol (4.26): To a solution of butane-1,4-diol (4.95 mL, 55.5 mmol) in dry THF (36 mL) was added NaH (50%, 2.66 g, 55.5 mmol) at 0 °C. Subsequently, BnBr (6.60 mL, 55.5 mmol) in THF (12 mL) was added to the mixture and a small amount of DMF to increase solubility. The resulting suspension was stirred overnight under argon at room temperature, after which the reaction was quenched with aq. NH_4Cl (sat.). The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were washed with brine (sat.), dried (MgSO_4) and concentrated. The product was purified by column chromatography (*n*-pentane-EtOAc 4:1 to 7:3) to give **4.26** (8.10 g, 44.9 mmol, 81%) as a light yellow oil. NMR-data were as reported in the literature.⁵²

3-Benzyloxy-propionaldehyde (4.27):⁵² To a solution of PCC (5.3 g, 24 mmol, 2.2 eq) in dichloromethane (38 mL) was added a solution of **4.26** (2.0 g, 11 mmol) in dichloromethane (6.8 mL). The resulting dark green mixture was stirred under argon overnight and then filtered over a silica plug (dichloromethane). After concentration, **4.27** (1.2 g, 6.7 mmol, 61%) was obtained as a colorless liquid, which was used without further purification. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ = 1.95 (m, 2H), 2.54 (dt, J = 1.6, 6.8, 14.4 Hz, 2H), 3.50 (t, J = 6.4 Hz, 2H), 4.49 (s, 2H), 7.24-7.37 (m, 5H), 9.77 (t, J = 1.6 Hz, 1H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100.6 MHz) δ = 22.3 (t), 40.7 (t), 68.9 (t), 72.7 (t), 127.4 (d), 128.1 (d), 138.0 (s), 202.0 (d) ppm.

S-Ethyl (2E)-6-(benzyloxy)hex-2-enethioate (4.28): Preparation of the Wittig reagent:⁴¹ Bromoacetic acid (13.9 g, 100 mmol), ethanethiol (9.65 mL, 130 mmol) and DMAP (1.22 g, 10.0 mmol) were dissolved in dichloromethane (450 mL) and cooled to 0 °C. To the resulting mixture was added DCC (21.7 g, 105 mmol) in three equal portions, after which the solution was warmed to room temperature and stirred overnight under argon. The resulting white suspension was filtered over celite and the residue was washed with dichloromethane (3x). The filtrate was partially concentrated in vacuo and then washed with aq. NaHCO_3 (sat.), water and brine (sat.). The organic layer was dried (Na_2SO_4), filtered and concentrated to give a yellow oil, which was dissolved in benzene (150 mL). To this solution was added PPh_3 (26.3 g, 100 mmol), after which the mixture was left to crystallize for 4 days. The crystals were filtered off and washed with cold toluene, after which they were dissolved in dichloromethane. The solution was washed with aq. Na_2CO_3 (10% w/w) and the aqueous layer was extracted with dichloromethane (2x). The combined organic

layers were dried (MgSO₄), filtered and partially concentrated. The product was crystallized from a mixture of dichloromethane and *n*-pentane to give EtSC(O)C=PPh₃ (25 g, 69 mmol, 69%) as white/colorless crystals. ¹H-NMR (CDCl₃, 400 MHz) δ = 1.25 (t, *J* = 7.6 Hz, 3H), 2.84 (q, *J* = 7.6 Hz, 2H), 3.66 (d, *J* = 21.6 Hz, 1H), 7.44-7.65 (m, 15H) ppm.

EtSC(O)C=PPh₃ (1.21 g, 3.33 mmol, 1.1 eq) was added to a solution of **4.27** (540 mg, 3.03 mmol) in chloroform (35 mL) and the resulting mixture was refluxed under argon for 3 h. The reaction mixture was cooled to room temperature and then stirred overnight in the presence of DMAP (100 mg, 0.82 mmol), after which the solvent was removed in vacuo. *Trans*-**4.28** (more polar, 697 mg, 2.64 mmol, 87%) and a small amount of *cis*-**4.28** (less polar) were obtained as colorless oils after purification by column chromatography (*n*-pentane-EtOAc 95:5 to 9:1). ¹H-NMR *trans*-**4.28** (CDCl₃, 400 MHz) δ = 1.28 (t, *J* = 7.6 Hz, 3H), 1.78 (m, 2H), 2.31 (m, 2H), 2.94 (q, *J* = 7.6 Hz, 2H), 3.49 (t, *J* = 6.4 Hz, 2H), 4.50 (s, 2H), 6.11 (d, *J* = 15.2 Hz, 1H), 6.90 (dt, *J* = 7.2, 15.2 Hz, 1H) 7.26-7.37 (m, 5H) ppm. ¹³C-NMR *trans*-**4.28** (CDCl₃, 100.6 MHz) δ = 14.6 (q), 22.9 (t), 27.9 (t), 28.7 (t), 69.0 (t), 72.8 (t), 127.42 (d), 127.44 (d), 128.2 (d), 128.8 (d), 138.2 (s), 144.3 (d) 189.9 (s) ppm. MS(EI) for C₁₅H₂₀O₂S-C₂H₅: *m/z* = 235 [M⁺].

¹H-NMR *cis*-**4.28** (CDCl₃, 400 MHz) δ = 1.28 (t, *J* = 7.6 Hz, 3H), 1.78 (m, 2H), 2.74 (m, 2H), 2.92 (q, *J* = 7.6 Hz, 2H), 3.50 (t, *J* = 6.4 Hz, 2H), 4.50 (s, 2H), 5.98-6.08 (m, 2H), 7.26-7.36 (m, 5H) ppm. ¹³C-NMR *cis*-**4.28** (CDCl₃, 100.6 MHz) δ = 14.6 (q), 23.1 (t), 26.7 (t), 29.1 (t), 69.6 (t), 72.8 (t), 126.5 (d), 127.4 (d), 127.5 (d), 128.2 (d), 138.3 (s), 146.0 (d) 189.7 (s) ppm. MS(EI) for C₁₅H₂₀O₂S-C₂H₅S: *m/z* = 203 [M⁺].

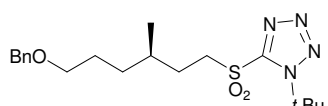
S-Ethyl (3R)-6-(benzyloxy)-3-methylhexanethioate (4.29):³³ (R,S)-Josiphos (L*, 67 mg, 0.11 mmol, 6 mol%) and CuBr·SMe₂ (19 mg, 0.094 mmol, 5 mol%) were dissolved in *t*BuOMe (17 mL) and stirred for 30 min under argon at room temperature. The mixture was cooled to -78 °C and MeMgBr (3.0 M in Et₂O, 0.78 mL, 2.3 mmol, 1.3 eq) was added dropwise over 2 min. After stirring for 10 min, a solution of *trans*-**4.28** (495 mg, 1.87 mmol) in *t*BuOMe (1.9 mL) was added over 5 h by syringe pump and the resulting mixture was stirred overnight at -78 °C. The reaction was quenched with MeOH at -78 °C, removed from the cold bath and diluted with H₂O, aq. NH₄Cl (sat.) and Et₂O. The product was extracted with Et₂O (3x), the combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. (3R)-**4.29** (485 mg, 1.73 mmol, 92%, 93% ee) was isolated as a colorless liquid after purification by column chromatography (*n*-pentane-EtOAc 95:5). (3R)-**4.29**: [α]_D²² = +5.1° (c = 1.88, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.94 (d, *J* = 6.8 Hz, 3H), 1.24 (t, *J* = 7.6 Hz, 3H), 1.22-1.31 (m, 1H), 1.41 (m, 1H), 1.63 (m, 2H), 1.94 (m, 1H), 2.36 (dd, *J* = 8.4, 14.4 Hz, 1H), 2.54 (dd, *J* = 5.6, 14.4 Hz, 1H), 2.87 (q, *J* = 7.2 Hz, 2H), 3.45 (t, *J* = 6.4 Hz, 2H), 4.50 (s, 2H), 7.26-7.37 (m, 5H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 14.7 (q), 19.3 (q) 23.2 (t), 27.0 (t), 30.8 (d), 32.9 (t), 51.2 (t), 70.3 (t), 72.8 (t), 127.4 (d), 127.5 (d), 128.2 (d), 138.4 (s), 199.1 (s) ppm. MS(EI) for C₁₆H₂₄O₂S: *m/z* = 280 [M⁺], HRMS calcd for C₁₆H₂₄O₂S: 280.150, found: 280.149. Ee determination by chiral HPLC analysis, chiralcel OD-H column, *n*-heptane-*i*PrOH 99.5:0.5, retention times: 20.77 (R) / 22.19 (S) min.

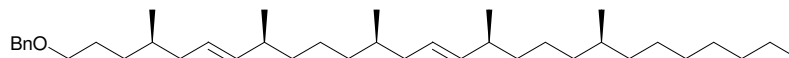
6-Benzyloxy-(3R)-3-methyl-hexan-1-ol (4.30): **4.29** (555 mg, 1.98 mmol) was dissolved in THF (6.0 mL) and LiAlH₄ (188 mg, 4.95 mmol, 2.5 eq) was added at 0 °C. The resulting suspension was stirred for 45 min at 0 °C under argon and then quenched with water. NaOH (3 M aq.) was added and the mixture was stirred until two clear layers were obtained. The layers were separated and the aqueous layer was extracted with Et₂O (3x). The combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. Purification by column chromatography (*n*-pentane-EtOAc 7:3) gave **4.30** (421 mg, 1.89 mmol, 96%) as a colorless liquid. (3R)-**4.30**: [α]_D²² = +4.9° (*c* = 1.10, CHCl₃), Lit. [α]_D⁵³ = +5.6°, ¹H-NMR (CDCl₃, 400 MHz) δ = 0.90 (d, *J* = 6.8 Hz, 3H), 1.22 (m, 1H), 1.40 (m, 2H), 1.54-1.73 (m, 3H), 3.46 (t, *J* = 6.4 Hz, 2H), 3.67 (m, 2H), 4.50 (s, 2H), 7.26-7.37 (m, 5H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 19.4 (q), 27.0 (t), 29.2 (d), 33.3 (t), 39.7 (t), 61.0 (t), 70.6 (t), 72.8 (t), 127.4 (d), 127.5 (d), 128.2 (d), 138.5 (s) ppm. MS(EI) for C₁₄H₂₂O₂: *m/z* = 222 [M⁺], HRMS calcd for C₁₄H₂₂O₂: 222.162, found: 222.164.

5-(6-Benzyloxy-(3R)-3-methyl-hexylthio)-1-tert-butyl-1H-tetrazole (4.31): Preparation of 1-tert-butyl-1H-tetrazole-5-thiol:⁵⁴ Sodium azide (5.64 g, 86.8 mmol) was dissolved in water (27 mL) and heated under reflux, after which a solution of *tert*-butylisothiocyanate (10.0 g, 86.8 mmol) in *i*-PrOH (21 mL) was added over 2 h. The resulting mixture was heated under reflux overnight and then cooled to 0 °C. Subsequently, concentrated aq. HCl (37%, 13 mL) was added and the solution was concentrated to 25-30 mL upon which a solid appeared. The suspension was stored at 0 °C for 5 h, after which the solid was filtered off. The residue was washed with ice-water and then crystallized from cyclohexane. The crystals were dried in vacuo at 50 °C for 5 h, after which recrystallization from cyclohexane afforded 1-*tert*-butyl-1H-tetrazole-5-thiol (8.85 g, 55.9 mmol, 64.4%) as a mixture of yellow and white crystals.

To a solution of PPh₃ (779 mg, 2.96 mmol, 1.5 eq) and **4.30** (440 mg, 1.98 mmol) in THF (12.0 mL) at 0 °C was added DIAD (0.68 mL, 3.5 mmol, 1.8 eq) over 2 min resulting in a yellow suspension. Subsequently, a solution of 1-*tert*-butyl-1H-tetrazole-5-thiol (626 mg, 3.96 mmol, 2.0 eq) in THF (6.0 mL) was added over 5 min and the reaction mixture was warmed to room temperature. After 1 h TLC showed complete conversion and the reaction was quenched with brine (sat.). The aqueous layer was extracted with Et₂O (3x) and the combined organic layers were dried (MgSO₄) and concentrated. Purification by column chromatography (*n*-pentane-EtOAc 9:1) gave **4.31** (610 mg, 1.68 mmol, 85%) as a colorless oil. (3R)-**4.31**: [α]_D²⁰ = -3.7° (*c* = 1.18, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.95 (d, *J* = 6.0 Hz, 3H), 1.24 (m, 1H), 1.43 (m, 1H), 1.54-1.70 (m, 3H), 1.71 (s, 9H), 1.71-1.86 (m, 2H), 3.31-3.50 (m, 2H), 3.45 (t, *J* = 6.4 Hz, 2H), 4.49 (s, 2H), 7.24-7.36 (m, 5H) ppm. ¹³C-NMR (CDCl₃, 50.32 MHz) δ = 19.0 (q), 27.0 (t), 28.5 (q), 31.8 (t), 32.1 (d), 32.8 (t), 35.8 (t), 60.7 (s), 70.4 (t), 72.8 (t), 127.3 (d), 127.5 (d), 128.2 (d), 138.4 (s), 152.6 (s) ppm. MS(EI) for C₁₉H₃₀N₄OS *m/z* = 362 [M⁺], HRMS calcd for C₁₉H₃₀N₄OS: 362.214, found: 362.213.

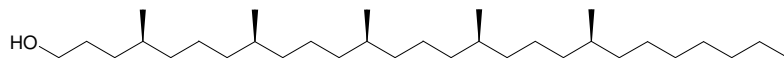
5-(6-Benzyloxy-(3R)-3-methyl-hexane-1-sulfonyl)-1-tert-butyl-1H-tetrazole (4.32): 4.31

 (610 mg, 1.68 mmol) was dissolved in dichloromethane (18.0 mL) and *m*CPBA (70%, 2.44 g, 9.90 mmol, 5.9 eq) was added at 0 °C. The resulting suspension was warmed to room temperature and stirred overnight. The reaction was quenched with aq. Na₂S₂O₃ (10% w/w) and diluted with dichloromethane. The organic layer was washed with aq. Na₂S₂O₃ (10% w/w, 3x; no more peroxides present) and the combined aqueous layers were subsequently extracted with dichloromethane (3x). The combined organic layers were washed with aq. NaHCO₃ (sat. 3x) and brine (sat.), dried (MgSO₄) and concentrated. The product was purified by column chromatography (*n*-pentane-EtOAc 9:1) to give **4.32** (631 mg, 1.60 mmol, 95%) as a colorless oil. (3R)-**4.32**: [α]_D²⁰ = -3.8° (c = 1.00, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.99 (d, *J* = 6.8 Hz, 3H), 1.30 (m, 1H), 1.47 (m, 1H), 1.55-1.73 (m, 3H), 1.80 (m, 1H), 1.85 (s, 9H), 1.99 (m, 1H), 3.47 (t, *J* = 6.4 Hz, 2H), 3.77 (ddd, *J* = 5.2, 10.8, 14.4 Hz, 1H), 3.84 (ddd, *J* = 5.2, 11.2, 14.4 Hz, 1H), 4.50 (s, 2H), 7.26-7.37 (m, 5H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 18.9 (q), 26.9 (t), 28.4 (t), 29.5 (q), 31.8 (d), 32.6 (t), 54.8 (t), 65.2 (s), 70.1 (t), 72.8 (t), 127.4 (d), 127.5 (d), 128.2 (d), 138.3 (s), 153.8 (s) ppm. MS(EI) for C₁₉H₃₀N₄O₃S *m/z* = 394 [M⁺], HRMS calcd for C₁₉H₃₀N₄O₃S-C₆H₅: 337.133, found: 337.133.



((4R,8S,12R,16S,20S)-4,8,12,16,20-Pentamethyl-heptacos-6,14-dienyloxymethyl)-benzene (4.33):

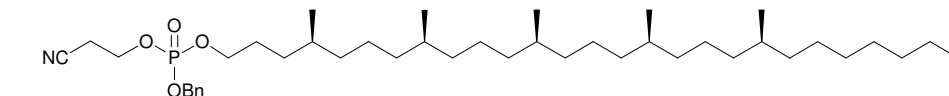
To a solution of **4.25** (455 mg, 1.25 mmol) and **4.32** (608 mg, 1.54 mmol, 1.24 eq) in THF (19 mL) at -78 °C was added LiHMDS (1.0 M in THF, 1.41 mL, 1.41 mmol, 1.13 eq) in a dropwise fashion over 5 min. The resulting yellow solution was stirred at -78 °C for 3 h and then allowed to warm to room temperature overnight. The reaction was quenched with aq. NH₄Cl (sat.) and the aqueous layer was extracted with Et₂O (3x). The combined organic layers were washed with brine (sat.), dried (MgSO₄) and concentrated. Purification by column chromatography (*n*-pentane-Et₂O 98:2) gave **4.33** (549 mg, 0.99 mmol, 80%) as a colorless oil. (4R,8S,12R,16S,20S)-**4.33**: [α]_D²² = +15.0° (c = 0.55, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.83 (d, *J* = 6.4 Hz, 6H), 0.87 (d, *J* = 6.4 Hz, 3H), 0.89 (t, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 6H), 1.00-1.50 (m, 29H), 1.63 (m, 2H), 1.81 (m, 2H), 1.93-2.09 (m, 4H), 3.45 (t, *J* = 6.8 Hz, 2H), 4.51 (s, 2H), 5.19-5.35 (m, 4H), 7.25-7.37 (m, 5H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 14.0 (q), 19.3 (q), 19.4 (q), 19.6 (q), 20.9 (q), 21.0 (q), 22.6 (t), 24.66 (t), 24.68 (t), 27.0 (t), 27.2 (t), 29.3 (t), 29.9 (t), 31.8 (t), 32.6 (d), 32.7 (t), 33.0 (d), 33.1 (d), 36.5 (t), 36.69 (d), 36.73 (d), 37.03 (t), 37.04 (t), 37.3 (t), 37.4 (t), 39.9 (t), 40.0 (t), 70.8 (t), 72.8 (t), 126.5 (d), 126.8 (d), 127.3 (d), 127.5 (d), 128.2 (d), 137.7 (d), 137.8 (d), 138.6 (s) ppm. MS(EI) for C₃₉H₆₈O *m/z* = 552 [M⁺], HRMS calcd for C₃₉H₆₈O: 552.527, found: 552.528.



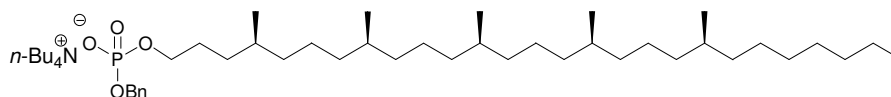
(4S,8S,12S,16S,20S)-4,8,12,16,20-Pentamethyl-heptacosan-1-ol (4.34): 4.33 (549 mg, 0.99 mmol) was dissolved in EtOAc (50 mL) and Pd/C (10%, 750 mg) was added. The resulting suspension was degassed with 3 vacuum-argon cycles and then saturated with H₂

by 5 vacuum-H₂ cycles. After stirring overnight under an H₂-atmosphere (1 bar), TLC showed complete conversion. The Pd/C was removed by filtration over a Florisil-plug and the resulting clear solution was concentrated in vacuo. After purification by column chromatography (*n*-pentane-EtOAc 9:1 to 9:2) **4.34** (381 mg, 0.82 mmol, 83%) was isolated as a colorless oil. (4*S*,8*S*,12*S*,16*S*,20*S*)-**4.34**: $[\alpha]_D^{22} = -0.8^\circ$ (*c* = 1.16, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.84-0.90 (m, 18H), 1.00-1.42 (m, 44H), 1.57 (m, 2H), 3.63 (t, *J* = 6.8 Hz, 2H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 14.0 (q), 19.6 (q), 19.7 (q), 22.6 (t), 24.4 (t), 27.0 (t), 29.3 (t), 29.9 (t), 30.3 (t), 31.8 (t), 32.5 (d), 32.7 (d), 32.8 (t), 37.0 (t), 37.3 (t), 63.4 (t) ppm. MS(EI) for C₃₂H₆₆O-H₂O *m/z* = 448 [M⁺], MS(CI) for C₃₂H₆₆O: *m/z* = 484 (M + NH₄)⁺, HRMS calcd for C₃₂H₆₆O-H₂O: 448.501, found: 448.501. GC-characterization: HP1-kolom, 30 m x 0.25 mm, He-flow = 1.0 mL/min, T_i = 70 °C for 70 min, T_f = 300 °C, rate 10 °C/min, rt. 79.8 min (1 peak only).

Benzyl 2-cyanoethyl *N,N*-diisopropylphosphoramidite (4.35):² Hunig's base (EtN(*i*Pr)₂, 1.47 mL, 8.44 mmol) was added to benzyl alcohol (0.44 mL, 4.27 mmol) and after stirring for 5 min, 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.0 g, 4.23 mmol) was added. The resulting mixture was stirred for 1 h at room temperature and was then diluted with dichloromethane and aq. NaHCO₃ (sat.). The aqueous layer was extracted with dichloromethane (2x) and the combined organic layers were dried (Na₂SO₄), filtered and concentrated. Purification by column chromatography (*n*-pentane-Et₂O-Et₃N 80:20:1) furnished **4.35** (829 mg, 2.69 mmol, 64%) as a colorless liquid. NMR-data were as reported in the literature:² ¹H-NMR (CDCl₃, 400 MHz) δ = 1.20 (t, *J* = 7.2 Hz, 12H), 2.62 (t, *J* = 6.4 Hz, 2H), 3.66 (m, 2H), 3.85 (m, 2H), 4.67 (dd, *J* = 8.8, 12.4 Hz, 1H), 4.76 (dd, *J* = 8.4, 12.4 Hz, 1H), 7.25-7.36 (m, 5H) ppm.

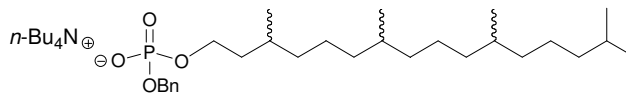


Benzyl-2-cyanoethyl-(4*S*,8*S*,12*S*,16*S*,20*S*)-4,8,12,16,20-pentamethyl-heptacosylphosphate (4.36): To an emulsion of **4.34** (76 mg, 0.16 mmol) and **4.35** (60 mg, 0.20 mmol, 1.2 eq) in acetonitrile (0.83 mL) was added ¹H-tetrazole (34 mg, 0.49 mmol, 3.0 eq) and the resulting mixture was stirred for 1.5 h. Subsequently, *tert*-butylhydroperoxide (5.0-6.0 M in decane, 0.16 mL, 0.80-0.96 mmol, 5.0-6.0 eq) was added in a dropwise fashion and stirring was continued for 1 h. The resulting clear solution was concentrated in vacuo and **4.36** (104 mg, 0.15 mmol, 92%) was obtained as a colorless oil after purification by column chromatography (*n*-pentane-EtOAc 4:1 to 2:1 to 1:1). (4*S*,8*S*,12*S*,16*S*,20*S*)-**4.36**: $[\alpha]_D^{22} = -0.2^\circ$ (*c* = 1.03, CHCl₃), ¹H-NMR (CDCl₃, 400 MHz) δ = 0.81-0.90 (m, 18H), 1.00-1.42 (m, 43H), 1.65 (m, 2H), 2.66 (dt, *J* = 2.8, 6.4 Hz, 2H), 3.03 (q, *J* = 6.8 Hz, 2H), 4.15 (m, 2H), 5.10 (d, *J* = 9.2 Hz, 2H), 7.35-7.40 (m, 5H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 14.0 (q), 19.3-19.6 (q + t), 22.6 (t), 24.4 (t), 27.0 (t), 27.6 (t), 27.7 (t), 29.3 (t), 29.9 (t), 31.8 (t), 32.3 (d), 32.4 (t), 32.6-32.7 (d), 36.9-37.3 (t), 61.4 (t, *J*_{C-P} = 4.6 Hz), 68.8 (t, *J* = 6.9 Hz), 69.6 (t, *J*_{C-P} = 5.3 Hz), 128.0 (d), 128.6 (d), 128.7 (d), 135.4 (s) ppm.



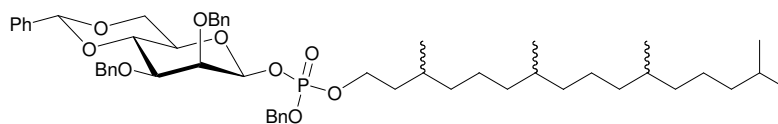
Tetrabutylammonium-benzyl-(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethyl-

heptacosylphosphate (4.37): To a solution of **4.36** (96 mg, 0.14 mmol) in dichloromethane (0.56 mL) was added a solution of tetra-*n*-butylammonium hydroxide (54 mg, 0.21 mmol, 1.5 eq) in water (0.56 mL) and the resulting mixture was stirred vigorously for 1 h. The reaction mixture was diluted with dichloromethane and water and the aqueous layer was extracted with dichloromethane (2x). The combined organic layers were dried (Na₂SO₄), filtered and concentrated to give **4.37** (122 mg, quantitative) as a yellow oil. ¹H-NMR (CDCl₃, 400 MHz) δ = 0.76-0.86 (m, 18H), 0.93 (t, *J* = 7.2 Hz, 12H), 0.93-1.42 (m, 51H), 1.59 (m, 10H), 3.30 (m, 8H), 3.79 (q, *J* = 6.4 Hz, 2H), 4.91 (d, *J* = 6.0 Hz, 2H), 7.14-7.40 (m, 5H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 13.5 (q), 14.0 (q), 19.3-19.6 (q + t), 22.5 (t), 23.9 (t), 24.3 (t), 26.9 (t), 28.4 (t), 28.5 (t), 29.2 (t), 29.8 (t), 31.7 (t), 32.58 (d), 32.61 (d), 33.0 (t), 33.1 (t), 36.9-37.4 (t), 58.6 (t), 65.3 (t), 66.4 (t), 126.5 (d), 127.1 (d), 127.7 (d) ppm.



Tetra-*n*-butylammonium-benzyl-3,7,11,15-tetramethyl-hexadecylphosphate (4.38):^{2,30g}

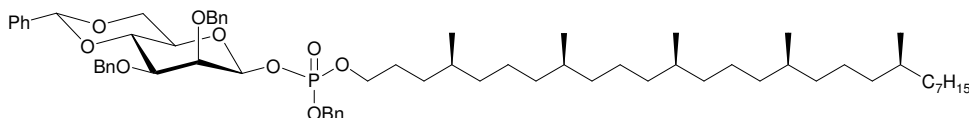
Prepared as described for the synthesis of **4.37**. The alcohol was obtained from commercial phytol *via* hydrogenation over PtO₂.^{30g} NMR-data were as reported in the literature: ¹H-NMR (CDCl₃, 400 MHz) δ = 0.80-0.86 (m, 15H), 0.97 (t, *J* = 7.2 Hz, 12H), 0.95-1.43 (m, 28H), 1.51 (m, 2H), 1.62 (m, 10H), 3.33 (m, 8H), 3.86 (m, 2H), 4.93 (d, *J* = 6.4 Hz, 2H), 7.14-7.45 (m, 5H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 13.6, 19.5-19.6, 22.5, 22.6, 24.0, 24.3, 24.4, 24.7, 27.9, 29.5, 32.7, 37.1-37.4, 39.2, 58.7, 63.3, 66.5, 126.6, 127.2, 127.7 ppm.



Benzyl 3,7,11,15-tetramethyl-hexadecylphosphoryl 2,3-di-*O*-benzyl-4,6-benzylidene-β-

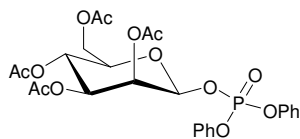
D-mannopyranoside (4.39):^{2,30g} To a solution of **4.3** (152 mg, 0.27 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP, 56 mg, 0.27 mmol) in toluene (11 mL) at -78 °C was added Tf₂O (55 μL, 0.33 mmol, 1.2 eq) in a dropwise fashion. The resulting mixture was stirred for 15 min under argon, after which a solution of **4.38** (581 mg, 0.82 mmol, 3.0 eq) in toluene (2.7 mL) was added. The mixture was stirred at -78 °C for 6 h and then quenched at this temperature with aq. NaHCO₃ (sat.). After warming to room temperature, the aqueous layer was extracted with EtOAc (2x) and the combined organic layers were washed with brine (sat.), dried (MgSO₄), filtered and concentrated. Because **4.39** was difficult to separate from the hydrolysis product of **4.3**, the crude product was dissolved in pyridine (1.8 mL), after which Ac₂O (0.2 mL) was added. The mixture was stirred overnight and then concentrated in vacuo. The remaining oil was dissolved in EtOAc and washed with aq. NaHCO₃ (sat.), aq. CuSO₄ (sat.) and brine (sat.). The organic layer was

dried (MgSO₄), filtered and concentrated. Purification by column chromatography (*n*-pentane-EtOAc 4:1 to 2:1) gave **4.39** (72 mg, 0.10 mmol, 37%, mixture of two diastereomers at P) as a yellow oil. NMR-data were as reported in the literature: ¹H-NMR (CDCl₃, 400 MHz) δ = 0.78-0.90 (m, 15H), 0.99-1.50 (m, 21H), 1.51 (m, 2H), 1.68 (m, 1H), 3.40 (m, 1H), 3.63 (2dd, *J* = 2.8, 10.0 Hz for both isomers, 1H), 3.81-4.15 (m, 4H), 4.20 (t, *J* = 9.6 Hz, 1H), 4.29 (dd, *J* = 4.8, 10.4 Hz, 1H), 4.61 + 4.62 (2d, isomer 1 *J* = 12.4 Hz, isomer 2 *J* = 12.0 Hz, 1H), 4.73 (d, *J* = 12.4 Hz, 1H), 4.77 (s, 1H), 4.89 (m, 1H), 5.05 (d, *J* = 8.0 Hz, 1H), 5.08 (m, 1H), 5.24 (brt, *J* = 6.8 Hz, 1H), 5.59 + 5.60 (2s, one for each isomer, 1H), 7.26-7.54 (m, 20H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 19.1, 19.2, 19.5, 19.6, 22.5, 22.6, 24.2, 24.4, 24.7, 27.8, 29.15, 29.18, 29.6, 32.7, 36.8-37.4, 39.2, 66.7, 66.77, 66.84, 66.9, 67.8, 68.0, 68.1, 69.2, 69.3, 69.38, 69.44, 69.9, 72.6, 75.1, 75.2, 76.3, 76.4, 77.4, 77.5, 77.8-77.9, 96.8, 96.9, 101.4, 125.9, 127.4-128.8, 128.9, 131.5, 137.2, 137.9 ppm.



Benzyl (4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethyl-heptacosylphosphoryl 2,3-di-O-benzyl-4,6-benzylidene- β -D-mannopyranoside (4.40): Sulfon **4.3** (36 mg, 64 μ mol) was coupled to phosphate salt **4.37** (127 mg, 0.14 mmol, 2.3 eq) using DTBMP (13 mg, 64 μ mol) and Tf₂O (12 μ l, 71 μ mol, 1.1 eq) in toluene (2.5 + 0.5 mL) to yield **4.40** (7.0 mg, 6.6 μ mol, 10%, mixture of two diastereomers at P). The reaction was performed as described for the preparation of **4.39**. ¹H-NMR (CDCl₃, 400 MHz) δ = 0.74-0.90 (m, 18H), 0.96-1.44 (m, 43H), 1.64 (m, 2H), 3.34-3.51 (m, 1H), 3.63 (m, 1H), 3.70-4.41 (m, 6H), 4.61 (m, 1H), 4.70 (m, 1H), 4.80 (m, 1H), 4.90 (m, 1H), 5.07 (m, 2H), 5.25 (m, 1H), 5.63 (s, 1H), 7.23-7.54 (m, 20H) ppm.

Diphenyl-(2,3,4,6-tetra-O-acetyl- β -D-mannopyranosyl)-phosphate (β -4.41):⁴⁴ To a solution of 2,3,4,6-tetra-O-acetyl-D-mannopyranose (1.90 g, 5.46 mmol) and DMAP (1.52 g, 12.4 mmol, 2.28 eq) in dichloromethane (32 mL) at room temperature was added a solution of diphenyl chlorophosphate (2.66 mL, 12.8 mmol, 2.35 eq) in dichloromethane (13 mL) by syringe pump over 1 h. After stirring the resulting solution under argon for 2 h, the



reaction mixture was diluted with dichloromethane and washed with cold water, cold aq. HCl (0.5 M) and cold aq. NaHCO₃ (sat.). The organic layer was dried (MgSO₄), filtered and concentrated to give **4.41** (2.50 g, 4.31 mmol, 79%) as a mixture of anomers (ratio α : β = 1:4). The pure β -anomer of **4.41** (0.98 g, 1.69 mmol, 31%) was obtained after purification by column chromatography (*n*-pentane-EtOAc 8:3 to 3:2) together with a mixed fraction of anomers (1.40 g, 2.41 mmol, 44%). β -**4.41** was stable in solution at room temperature (even in chloroform) and for several months at -18°C when concentrated. However, when stored at room temperature after concentration, we observed that the fraction of α -anomer increased either by epimerization or by selective degradation of the β -anomer. ¹H-NMR β -**4.41** (CDCl₃, 400 MHz) δ = 1.94 (s, 3H, Ac), 2.00 (s, 6H, Ac), 2.05 (s, 3H, Ac), 3.77 (m, 1H, C5-H), 4.07 (dd, *J* = 2.4, 12.4 Hz, 1H, C6-H), 4.23 (dd, *J* = 5.6, 12.4 Hz, 1H, C6-H'),

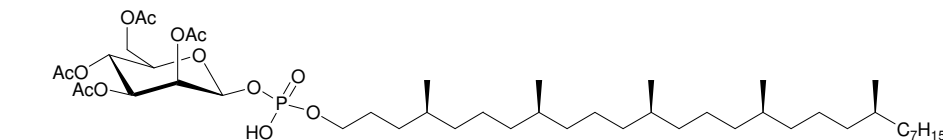
5.07 (dd, $J = 3.6, 9.6$ Hz, 1H, C3-H), 5.22 (t, $J = 9.6$ Hz, 1H, C4-H), 5.46 (d, $J = 3.2$ Hz, 1H, C2-H), 5.61 (d, $J = 6.8$ Hz, 1H, C1-H), 7.12-7.32 (m, 10H) ppm. ^{13}C -NMR (CDCl_3 , 100.6 MHz) $\delta = 20.2$ (q), 20.3 (q), 20.37 (q), 20.38 (q), 61.7 (t), 65.0 (d), 67.8 (d, $J_{\text{C-P}} = 8.5$ Hz), 69.8 (d), 72.7 (d), 94.5 (d, $J_{\text{C-P}} = 4.6$ Hz), 120.0 (d, $J_{\text{C-P}} = 4.5$ Hz), 120.1 (d, $J_{\text{C-P}} = 4.5$ Hz), 125.4 (d), 125.6 (d), 129.4 (d), 129.6 (d), 149.7 (s, $J_{\text{C-P}} = 7.6$ Hz), 150.1 (s, $J_{\text{C-P}} = 8.5$ Hz), 169.3 (s), 169.4 (s), 169.5 (s) 170.3 (s) ppm.

Diphenyl-(2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-phosphate (α -4.41):⁴⁴ A solution of DMAP (0.80 g, 6.6 mmol, 2.3 eq) and diphenyl chlorophosphate (1.4 mL, 6.8 mmol) in dichloromethane (6.7 mL) was added over 30 min to a solution of 2,3,4,6-tetra-*O*-acetyl-D-mannopyranose (1.00 g, 2.87 mmol) in dichloromethane (16.7 mL) at -30°C . The resulting mixture was stirred under argon for 1 h at -30°C and then maintained for 2 h between -10 and 0°C . Work-up and purification was performed as described for β -4.41 to give α -4.41 (1.09 g, 1.88 mmol, 65%) as a colorless oil. ^1H -NMR (CDCl_3 , 400 MHz) $\delta = 1.95$ (s, 3H, Ac), 1.98 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.13 (s, 3H, Ac), 3.89 (dd, $J = 2.0, 12.4$ Hz, 1H, C6-H), 4.05 (m, 1H, C5-H), 4.16 (dd, $J = 4.8, 12.4$ Hz, C6-H'), 5.23-5.37 (m, 3H, C2-H, C3-H and C4-H), 5.85 (dd, $J = 2.0, 6.8$, 1H, C1-H), 7.18-7.37 (m, 10H) ppm. ^{13}C -NMR (CDCl_3 , 100.6 MHz) $\delta = 20.30$ (q), 20.31 (q), 20.36 (q), 20.41 (q), 61.4 (t), 64.8 (d), 67.9 (d), 68.4 (d, $J_{\text{C-P}} = 12.3$ Hz), 70.5 (d), 95.9 (d, $J_{\text{C-P}} = 5.3$ Hz), 119.8 (d, $J_{\text{C-P}} = 4.6$ Hz), 120.0 (d, $J_{\text{C-P}} = 4.5$ Hz), 125.6 (d), 125.7 (d), 129.7 (d), 129.8 (d), 149.8 (s, $J_{\text{C-P}} = 6.9$ Hz), 150.0 (s, $J_{\text{C-P}} = 6.8$ Hz), 169.3 (s), 169.5 (s), 170.3 (s) ppm.

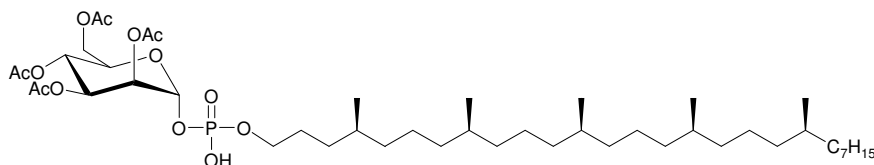
Pyridinium (2,3,4,6-tetra-*O*-acetyl- β -D-mannopyranosyl)-phosphate (β -4.42): β -4.41 (667 mg, 1.15 mmol) was dissolved in EtOH-EtOAc (14.4 mL, 1:1 v/v) and PtO_2 (29 mg, 0.13 mmol, 0.11 eq) was added. The resulting suspension was degassed with 3 vacuum-argon cycles and then saturated with H_2 by 5 vacuum- H_2 cycles. After stirring over for 60 h under an H_2 -atmosphere (1 bar), TLC (chloroform/MeOH/water 60/35/6 v/v/v) showed complete conversion. The catalyst was removed by filtration over celite and the solution was neutralized with pyridine. After concentration β -4.41 (469 mg, 0.92 mmol, 80%) was isolated as a white foam. ^1H -NMR (D_2O , 400 MHz) $\delta = 1.88$ (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.99 (s, 3H, Ac), 2.10 (s, 3H, Ac), 3.93 (ddd, $J = 2.0, 3.6, 10.0$ Hz, 1H, C5-H), 4.07 (dd, $J = 2.0, 12.8$ Hz, 1H, C6-H), 4.31 (ddd, $J = 3.6, 12.8$ Hz, 1H, C6-H'), 5.09 (t, $J = 10.0$ Hz, 1H, C4-H), 5.21 (dd, $J = 3.2, 10.4$ Hz, 1H, C3-H), 5.33 (dd, $J = 0.8, 8.8$ Hz, 1H, C1-H), 5.40 (d, $J = 2.8$ Hz, 1H, C2-H), 7.95 (t, $J = 7.2$ Hz, 2H, Py), 8.50 (m, 1H, Py), 8.65 (d, $J = 5.2$ Hz, 2H, Py) ppm. ^{13}C -NMR (D_2O , 50.32 MHz) $\delta = 18.6$ (q), 60.3 (t), 64.0 (d), 68.5 (d, $J_{\text{C-P}} = 6.8$ Hz), 69.6 (d), 70.3 (d), 91.8 (d), 125.9 (d), 171.1 (s), 171.6 (s), 171.7 (s), 172.2 (s) ppm. Positive ion ESI-HRMS calcd for $\text{C}_{14}\text{H}_{21}\text{O}_{13}\text{NaP}$ ($\text{M-C}_5\text{H}_6\text{N}^+ + \text{Na}^+ + \text{H}^+$): 451.062, found: 451.062.

Pyridinium (2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-phosphate (α -4.41

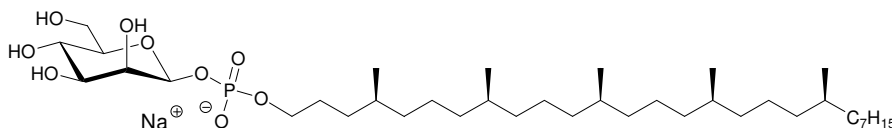
(1.0 g, 1.7 mmol) was converted into α -4.42 (831 mg, 1.64 mmol, 95%) using a procedure analogous to the synthesis of β -4.42. ¹H-NMR (D₂O, 400 MHz) δ = 1.92 (s, 3H, Ac), 2.00 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.10 (s, 3H, Ac), 4.07 (d, J = 12.8, 1H, C6-H), 4.28 (d, J = 10.0 Hz, 1H, C5-H), 4.36 (dd, J = 2.8, 12.8 Hz, 1H, C6-H'), 5.20 (t, J = 10.0 Hz, 1H, C4-H), 5.23 (brs, 1H, C2-H), 5.32 (dd, J = 3.2, 10.0 Hz, 1H), 5.40 (d, J = 8.0 Hz, 1H, C1-H), 7.99 (t, J = 7.2 Hz, 2H, Py), 8.50 (m, 1H, Py), 8.69 (d, J = 5.6 Hz, 2H, Py) ppm. ¹³C-NMR (D₂O, 50.32 MHz) δ = 18.6 (q), 60.4 (t), 64.0 (d), 67.3 (d), 67.9 (d), 68.3 (d, J_{C-P} = 10.3 Hz), 91.6 (d, J = 4.6 Hz), 125.9 (d), 171.1 (s), 171.3 (s), 171.7 (s), 172.2 (s) ppm.



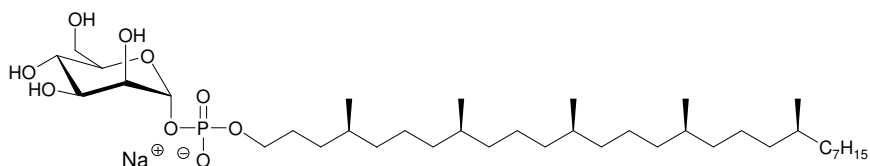
(4S,8S,12S,16S,20S)-4,8,12,16,20-Pentamethylheptacosylphosphoryl-2,3,4,6-tetra-*O*-acetyl- β -D-mannopyranoside (β -4.43): β -4.42 (129 mg, 0.25 mmol, 2.0 eq) was brought into a dry two-neck flask (50 mL) and co-evaporated with freshly distilled dry toluene (2x; pressure equalized with nitrogen). Subsequently a solution of 4.34 (59 mg, 0.13 mmol) in dry toluene was added and the mixture was co-evaporated with dry toluene (2x). Finally TPSCl (115 mg, 0.38 mmol, 3.0 eq) was added and after two more additions and evaporations of dry toluene, the residue was dissolved in dry pyridine (2.7 mL) and stirred under argon for 4 days. The reaction was quenched with MeOH (2.5 mL) and stirred for 2 more h before concentration (co-evaporation with toluene). The residue was dissolved in chloroform and washed with water (3x) and brine (sat.), dried (MgSO₄), filtered and concentrated. The product was purified by column chromatography (chloroform-MeOH 95:5 to 85:15) giving β -4.43 (90 mg, 0.10 mmol, 79%) as a white foam. (4S,8S,12S,16S,20S)- β -4.43: $[\alpha]_D^{22}$ = -9.7° (c = 1.22, CHCl₃), ¹H-NMR (CD₃OD + 1 drop of CDCl₃, 400 MHz) δ = 0.82-0.92 (m, 18H), 1.03-1.46 (m, 43H), 1.61 (m, 2H), 1.95 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.16 (s, 3H, Ac), 3.86 (m, 3H, C5-H and CH₂-OP), 4.16 (dd, J = 2.4, 12.4 Hz, 1H, C6-H), 4.28 (dd, J = 4.4, 12.0 Hz, 1H, C6-H'), 5.17-5.25 (m, 2H, C3-H, C4-H), 5.42 (d, J = 8.8 Hz, 1H, C1-H), 5.48 (brs, 1H, C2-H) ppm. ¹³C-NMR (CDCl₃-CD₃OD 1:1 (v/v), 100.6 MHz) δ = 14.3 (q), 19.7-20.0 (q), 20.7 (q), 20.8 (q), 23.0 (t), 24.8-24.9 (t), 27.4 (t), 28.5 (t), 28.6 (t), 29.7 (t), 30.3 (t), 32.3 (t), 33.1-33.2 (d), 33.4 (t), 33.5 (t), 37.4 (t), 37.5 (t), 37.8-37.9 (t), 62.6 (t), 66.0 (d), 67.1 (t), 70.1 (d, J_{C-P} = 6.1 Hz), 71.5 (d), 72.8 (d), 94.0 (d), 170.5 (s), 170.6 (s), 171.2 (s), 171.5 (s) ppm. ³¹P-NMR (CDCl₃-CD₃OD 1:1 (v/v), 161.6 MHz) δ = -5.6 ppm. Positive ion ESI-HRMS calcd for C₄₆H₈₅O₁₃NaP (M + Na)⁺: 899.563, found: 899.559.



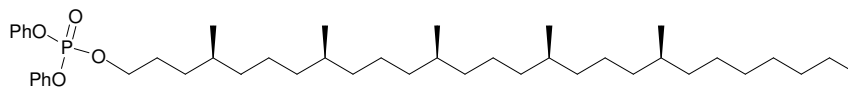
(4S,8S,12S,16S,20S)-4,8,12,16,20-Pentamethylheptacosylphosphoryl-2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (α -4.43): α -4.42 (110 mg, 0.21 mmol, 2.0 eq) was coupled to 4.34 (50 mg, 0.11 mmol, 1.0 eq) in the presence of TPSCl (98 mg, 0.32 mmol, 3.0 eq) to give α -4.43 (49 mg, 56 μ mol, 52%) using a procedure analogous to the synthesis of β -4.43. $^1\text{H-NMR}$ (CD_3OD + 1 drop of CDCl_3 , 400 MHz) δ = 0.82-0.92 (m, 18H), 1.03-1.46 (m, 43H), 1.61 (m, 2H), 1.96 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.15 (s, 3H, Ac), 3.89 (m, 2H, $\text{CH}_2\text{-OP}$), 4.11 (dd, J = 2.4, 12.0 Hz, 1H, C6-H), 4.22 (m, 1H, C5-H), 4.29 (dd, J = 4.0, 12.4 Hz, 1H, C6-H'), 5.28-5.37 (m, 3H, C2-H, C3-H and C4-H), 5.49 (d, J = 6.8 Hz, 1H, C1-H) ppm. $^{13}\text{C-NMR}$ ($\text{CDCl}_3\text{-CD}_3\text{OD}$ 1:1 (v/v), 100.6 MHz) δ = 14.5 (q), 20.0-20.3 (q), 20.7 (q), 20.81 (q), 20.85 (q), 23.4 (t), 25.2-25.3 (t), 27.8 (t), 29.1 (t), 29.2 (t), 30.2 (t), 30.7 (t), 32.7 (t), 33.5-33.6 (d), 33.9 (t), 37.8 (t), 37.9 (t), 38.15-38.23 (t), 63.1 (t), 66.5 (d), 67.4 (t), 70.0 (d), 70.3 (d), 70.6 (d, $J_{\text{C-P}}$ = 9.2 Hz), 94.5 (d), 170.99 (s), 171.02 (s), 171.1 (s), 171.9 (s) ppm. $^{31}\text{P-NMR}$ ($\text{CDCl}_3\text{-CD}_3\text{OD}$ 1:1 (v/v), 161.6 MHz) δ = -5.8 ppm. Negative ion ESI-HRMS calcd for $\text{C}_{46}\text{H}_{84}\text{O}_{13}\text{P}^-$ (M-H) $^-$: 875.566, found: 875.563.



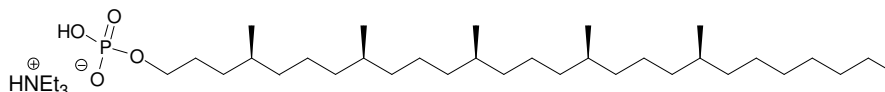
Sodium (4S,8S,12S,16S,20S)-4,8,12,16,20-Pentamethylheptacosylphosphoryl- β -D-mannopyranoside (β -4.1): β -4.43 (130 mg, 0.15 mmol) was dissolved in chloroform-MeOH (3.5 mL, 1:2.5 v/v) and NaOMe was added (14 mg). The resulting solution was stirred for 60 min and then diluted with 1-butanol (30 mL) and quenched with aq. NH_4Cl (sat. 20 mL). The organic layer was washed with water (3 x 20 mL) and then concentrated by co-evaporation with toluene. The resulting solid was placed on a pad of Celite and washed with acetone (50 mL) and then chloroform-MeOH (70 mL, 1:1 v/v). The chloroform-MeOH extract was concentrated to give β -4.1 (108 mg, 0.15 mmol, quantitative) as a white solid. (4S,8S,12S,16S,20S)- β -4.1: $[\alpha]_{\text{D}}^{22}$ = -3.6 $^\circ$ (c = 0.55, $\text{CHCl}_3\text{-CH}_3\text{OH-H}_2\text{O}$ 95:95:10 v/v/v), mp = 125 $^\circ\text{C}$ (decomp.), $^1\text{H-NMR}$ ($\text{CD}_3\text{OD-CDCl}_3\text{-D}_2\text{O}$ 0.45:0.45:0.05 (v/v/v), 400 MHz) δ = 0.82-0.88 (m, 18H), 1.00-1.42 (m, 43H), 1.61 (m, 2H), 3.28-3.32 (m, 1H, C5-H), 3.52-3.59 (m, 2H, C3-H and C4-H), 3.73 (dd, J = 5.6, 12.0 Hz, 1H, C6-H), 3.82-3.88 (m, 3H, C6-H' and $\text{CH}_2\text{-OP}$), 3.93 (d, J = 2.4 Hz, 1H, C2-H), 5.07 (d, J = 8.0 Hz, 1H, C1-H) ppm. $^{13}\text{C-NMR}$ ($\text{CD}_3\text{OD-CDCl}_3\text{-D}_2\text{O}$ 0.45:0.45:0.05 (v/v/v), 125.7 MHz) δ = 14.5 (q), 19.9 (q), 20.2-20.3 (q), 23.3 (t), 25.0 (t), 25.1 (t), 25.2 (t), 25.3 (t), 27.7 (t), 28.96 (t), 29.02 (t), 30.0 (t), 30.6 (t), 32.6 (t), 33.3-33.5 (d), 33.8 (t), 33.9 (t), 37.6 (t), 37.7 (t), 38.0-38.2 (t), 61.8 (t), 67.3 (d), 67.4 (t, $J_{\text{C-P}}$ = 6.2 Hz), 72.1 (d), 74.0 (d), 77.9 (d), 96.3 (d) ppm. $^{31}\text{P-NMR}$ ($\text{CD}_3\text{OD-CDCl}_3\text{-D}_2\text{O}$ 0.45:0.45:0.05 (v/v/v), 161.6 MHz) δ = -1.6 ppm. Negative ion ESI-HRMS calcd for $\text{C}_{38}\text{H}_{76}\text{O}_9\text{P}^-$ (M- Na^+) $^-$: 707.523, found: 707.524.



Sodium (4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosylphosphoryl- α -D-mannopyranoside (α -4.1): α -4.43 (42 mg, 48 μ mol) was converted into α -4.1 (35 mg, 48 μ mol, quantitative) as described for the preparation of β -4.1. $^1\text{H-NMR}$ ($\text{CD}_3\text{OD-CDCl}_3\text{-D}_2\text{O}$ 0.45:0.45:0.05 (v/v/v), 400 MHz) δ = 0.82-0.88 (m, 18H), 0.98-1.42 (m, 43H), 1.61 (m, 2H), 3.58-3.65 (m, 2H), 3.71 (dd, J = 5.6, 11.2 Hz, 1H, C6-H), 3.75-3.85 (m, 4H), 3.90 (m, 1H) 5.38 (dd, J = 1.6, 7.6 Hz, 1H, C1-H) ppm. $^{13}\text{C-NMR}$ ($\text{CD}_3\text{OD-CDCl}_3\text{-D}_2\text{O}$ 0.45:0.45:0.05 (v/v/v), 125.7 MHz) δ = 14.4 (q), 14.5 (q), 19.8 (q), 19.9 (q), 20.1-20.3 (q), 23.3 (t), 25.0 (t), 25.1-25.3 (t), 27.6 (t), 28.9 (t), 29.0 (t), 30.0 (t), 30.5 (t), 32.5 (t), 33.3-33.4 (d), 33.7 (t), 33.8 (t), 37.6 (t), 37.7 (t), 38.0 (t), 61.7 (t), 67.1 (t, $J_{\text{C-P}}$ = 5.3 Hz), 67.5 (d), 71.0 (d), 71.6 (d, $J_{\text{C-P}}$ = 8.4 Hz), 74.5 (d), 77.9 (d), 96.6 (d, $J_{\text{C-P}}$ = 5.4 Hz) ppm. $^{31}\text{P-NMR}$ ($\text{CD}_3\text{OD-CDCl}_3\text{-D}_2\text{O}$ 0.45:0.45:0.05 (v/v/v), 161.6 MHz) δ = -1.8 ppm. Negative ion ESI-HRMS calcd for $\text{C}_{38}\text{H}_{76}\text{O}_9\text{P}^-$ (M-Na^+): 707.523, found: 707.521.



(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosyl diphenyl phosphate (4.44): DMAP (30 mg, 0.25 mmol, 2.3 eq) was added to a solution of **4.34** (50 mg, 0.11 mmol) in dichloromethane (0.63 mL) and the resulting mixture was cooled to 0 °C. Subsequently, a solution of diphenyl chlorophosphate (51 μ L, 0.25 mmol, 2.3 eq) in dichloromethane (0.26 mL) was added over 5 min. The solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted with dichloromethane and then washed with water, aq. HCl (1.0 M), aq. NaHCO_3 (sat.) and brine (sat.). The organic layer was dried (MgSO_4), filtered and concentrated. Purification by column chromatography (*n*-pentane-EtOAc 95:5 to 92.5:7.5) yielded **4.44** (75 mg, 0.11 mmol, quantitative) as a colorless oil. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ = 0.81-0.95 (m, 18H), 1.01-1.44 (m, 43H), 1.71 (m, 2H), 4.25 (q, J = 6.8 Hz, 2H), 7.19 (t, J = 7.2 Hz, 1H), 7.25 (m, 2H), 7.35 (m, 2H) ppm. $^{13}\text{C-NMR}$ (CDCl_3 , 100.6 MHz) δ = 14.0 (q), 19.4 (q), 19.6 (q), 22.6 (t), 24.3 (t), 24.4 (t), 27.0 (t), 27.65 (t), 27.72 (t), 29.3 (t), 29.9 (t), 31.8 (t), 32.25 (d), 32.34 (t), 32.4 (t), 32.7 (d), 36.9 (t), 37.1 (t), 37.3 (t), 69.7 (t, $J_{\text{C-P}}$ = 6.9 Hz), 119.9 (d, J = 5.3 Hz), 125.1 (d), 129.6 (d), 150.6 (s) ppm. $^{31}\text{P-NMR}$ (CDCl_3 , 161.6 MHz) δ = -12.1 ppm.



Triethylammonium-(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosylphosphate (4.37): Alcohol **4.44** (70 mg, 0.10 mmol) was dissolved in EtOH-EtOAc (3.0 mL, 1:1 v/v) and PtO_2 (5 mg, 22 μ mol, 0.22 eq) was added. The resulting suspension was degassed with 3 vacuum-argon cycles and then saturated with H_2 by 5 vacuum- H_2 cycles. After stirring for 60 h under an H_2 -atmosphere (1 bar), $^1\text{H-NMR}$ showed

complete conversion. The catalyst was removed by filtration over celite and the solution was neutralized with triethylamine. After concentration **4.44** (65 mg, 0.10 mmol, quantitative) was isolated as a yellow oil. ¹H-NMR (CDCl₃, 400 MHz) δ = 0.78-0.90 (m, 18H), 0.96-1.40 (m, 44H), 1.29 (t, *J* = 7.2 Hz, 9H), 1.59 (m, 2H), 3.03 (q, *J* = 7.2 Hz, 6H), 3.84 (m, 2H) ppm. ¹³C-NMR (CDCl₃, 100.6 MHz) δ = 0.9 (q), 8.8 (q), 14.0 (q), 19.6 (q), 22.5 (t), 24.4 (t), 26.9 (t), 28.5 (t), 29.2 (t), 29.8 (t), 31.8 (t), 32.6 (d), 33.0 (t), 36.9 (t), 37.3 (t), 45.3 (t), 65.3 (t) ppm. ³¹P-NMR (CDCl₃, 161.6 MHz) δ = 1.8 ppm.

4.10 References

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